Longitudinal analysis techniques in epidemiology: how prospective cohort data can be used to understand pathways to cardiometabolic disease outcomes

Findings from the Cardiovascular Risk in Young Finns Study

by

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DECLARATION OF ORIGINAL AUTHORSHIP

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Marie-Jeanne BUSCOT
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THESIS ABSTRACT

The overall contribution of this thesis has been the application and development of growth modeling methods for analysing continuous longitudinal data arising from long-term observational studies in epidemiology, with a special emphasis on how these techniques can be used to investigate pathways leading to important cardiometabolic risk factors or deleterious cardiovascular disease outcomes in adulthood. These risk factors (obesity, dyslipidemia and hypertension) and clinical outcomes (pre-atherosclerosis, and type 2 diabetes mellitus) are important because they are associated with increased risk of developing heart disease and stroke.

This general aim was addressed in three parts:

1. First, analytical strategies and key issues relative to the application of growth curve modeling methods to continuous non-linear response data were identified, reviewed and compiled. In this phase, specific emphasis was placed on the technical considerations of data arising from cohort sequential studies where there were less than 10 time points per person collected across the life course, and where the time between sequential measures is not balanced between participants. This aim was addressed in Chapter 2, detailed below.
2. Second, growth curve modeling theory was tailored and extended to develop a Bayesian "trajectory divergence" method in Chapter 4. This method allows the identification of the point or age, in the life course when participants who develop important health outcomes later in life begin to diverge in their non-linear trajectories of continuous modifiable risk factors, compared with those who remain healthy.

3. Third, the reviewed and developed growth curve methodologies in part one were applied to model and analyse the longitudinal trajectories of a number of continuous measures of cardiometabolic risk. Data from the Cardiovascular Risk in Young Finns Study (YFS) was used. The Young Finns Study is an accelerated prospective cohort study that has collected cardiometabolic risk factor data in a large sample of Finnish participants from childhood to mid-adulthood across eight waves of follow-up over the course of 31 years. These analyses were written as a series of 4 original papers included in Chapters 3 to 6, which have been (or will be) submitted to journals.

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Chapter 2, the literature review, provides the background and foundation for the methodological component of the thesis. This chapter notably reviews traditional person-based growth modeling methods as well as key aspects to be considered when deciding to implement them in the multilevel (MLM) or in the latent growth curve (LGCM) modeling framework. It introduces the implementation and model building strategies of different complex growth models in the frequentist and Bayesian framework, with particular emphasis on analytical challenges that accompany the analysis of data from accelerated longitudinal study designs. In addition to person-based growth modeling approaches, it reviews an alternative approach to growth modeling, also known as Latent Class or group-based methods, and highlights how they can be used complementarily to traditional trajectory modeling approaches. By showing the unaddressed issues and difficulties when analysing scarce longitudinal data, this chapter motivates the research and the contribution that the thesis sought to make.

Chapter 3 addresses research aims 1 and 2 of the thesis. This chapter demonstrate how frequentist IGC modeling, a person-based approach to trajectory analyses can be used to investigate associations between longitudinal response profiles of complex

shapes and important individual cardiometabolic risk marker (in this case, genetic factors). Its main aim was to quantify for the first time the contribution of a multigenic lipoprotein score to adverse levels of circulating lipoproteins, important risk factors for cardiovascular disease in the YFS cohort. It was found that for LDL-C, HDL-C, and triglycerides, the polygenic effect of GWAS-identified risk alleles was present from early childhood (from age 3 years). In addition, it was shown that while the polygenic effect on LDL-C levels remained and consistent across the life course as YFS participants aged, the multigenic effect on HDL-C levels became weaker as participants aged, with environmental, lifestyle and behavioral factors such as diet, smoking, physical activity, potentially becoming more important determinants of HDL-C levels in mid-adulthood.

Chapter 4 formulates and develops a Growth curve modeling methodology based on Bayesian hierarchical piecewise regression (BHPR) to investigate trajectory divergence in non-linear continuous response profiles between two or more groups of participants in cohort studies and to determine when groups of interest begin to show different non-linear trajectories in continuous response data. To represent the curvilinear development of BMI between age 6 and 49 years, this method uses a two-piece linear–linear spline model as the functional form of the MLM growth curve model. The application of this novel Bayesian regression analysis method is then outlined by modeling individual BMI trajectories in a subset of YFS participants, showing that the participants who developed adult T2DM in adulthood, a major metabolic disease, maintain their childhood BMI rate for up to 12 years longer, compared with those who did not develop T2DM. In a simulation study, the age and confidence interval at divergence is estimated and compared to more traditional approaches. A simulation study demonstrates that the developed approach is superior to traditional trajectory divergence approaches commonly used used by epidemiologists.

Chapter 5 uses the BHPR approach developed in Chapter 4 and extends it to suit the estimation of a multiple-group growth model to estimate and compare developmental BMI trajectories in groups of clinical interest in the YFS. These groups were four *a-priori* determined child to adult adiposity status groups the YFS; participants who either remained obese, resolved obesity, remained non-obese, or became obese based on their BMI status between two YFS follow-up examinations: one in childhood (baseline study) and the latest available individual BMI assessment in adulthood. It was shown that participants who overcame childhood high-BMI by adulthood had in fact lower average BMI levels from age 6 and slower yearly increase in BMI in childhood compared with participants who remained obese adults, confirming that efforts to reverse adverse BMI trajectories should ideally begin before age 6 years. The method allowed identifying critical age windows for the development and resolution of adverse adiposity status in this large cohort, which is crucial to help understand the dynamics of pathological vs. normal BMI trajectories across developmental periods, since its potential to inform weight management interventions.

Chapter 6 investigates potential heterogeneity in BMI development in the YFS population from a life course perspective using Latent Class Growth Mixture Modeling, to determine whether distinct long-term BMI developmental patterns result in different cardiovascular risk in adulthood in the YFS. It was hypothesised that different BMI trajectory patterns exist within the YFS population, which could not be identified using the traditional person-based growth modeling approaches (such as the BHPR method). The analyses revealed that 6 subgroups shared different trajectories in BMI from childhood though middle age in the YFS, and that higher BMI trajectories were associated with higher adult cardiovascular risk factors of T2DM, dyslipidaemia, hypertension (high blood pressure) and preclinical atherosclerosis, a condition considered as the major underlying cause of cardiovascular disease.

Chapter 7, the discussion chapter, puts in perspective how the reviewed, developed state-of-the-art growth curve modeling approaches applied in the four original studies can be used to characterise longitudinal trajectories in cardiometabolic risk factors and their determinants, as well as potential heterogeneity that may arise in these trajectories within a population or groups of interest. The benefits, drawbacks and remaining technical challenges that come with applying these approaches to scarce long-term epidemiological data is discussed.

In conclusion, this thesis demonstrates the potential of sate of the art and tailored growth curve methods for the analysis of long term observational studies on cardiometabolic risk, and how they can be used to provide unique insight into the development of high-risk cardiometabolic profiles from childhood to adulthood that might help inform prevention and intervention strategies.

CONTENTS

STATEMENT OF AUTHORSHIP

The data used in this thesis comes the Cardiovascular Risk in Young Finns study (YFS), a large ongoing Finnish population-based prospective cohort of cardiovascular risk factors and their determinants. The data collection was complete when Marie-Jeanne Buscot (MJB) enrolled in her PhD program and therefore she was not involved in the study design or the data collection. MJB designed the research questions covered in this thesis based on the data available, and had sole responsibility for cleaning and preparing the data used in the different analyses.

This thesis includes papers for which MJB is not the sole author. MJB took the lead in this research in that she designed the research, cleaned and analysed the data used to address research aims in the different chapters and wrote the manuscripts. However, she was assisted by a number of co-authors. The contributions of each author are detailed below.

The paper reported in Chapter 3

The combined effect of common genetic risk variants on circulating lipoproteins is evident in childhood: a longitudinal analysis of the YFS Study.

Marie-Jeanne Buscot as first author was responsible for the data cleaning, statistical analysis, drafting of the manuscript and coordination of the manuscript revisions. Dr. Costan Magnussen and Dr. Russell Thomson were responsible for general advice regarding the study and editorial comments. Markus Juonala, Niina Pitkänen, Terho Lehtimäki, Jorma S.A. Viikari, Mika Kähönen, Nina Hutri-Kähönen collected the clinical data and biological samples used in this study. Niina Pitkänen processed the blood samples for sequencing. Olli T. Raitakari as the principal investigator of the YFS study contributed to early conceptual discussions related to this work and retained an overall advisory and editorial role. Nicholas J. Schork and all other listed authors reviewed and approve the final version of manuscript.

The paper reported in Chapter 4

Bayesian hierarchical piecewise regression models: A tool to detect trajectory divergence between groups in long-term observational studies.

Marie-Jeanne Buscot as first author was responsible for the concept of the paper, data analysis, interpretation, writing all drafts and addressing the reviewers' comments. Dr. Costan Magnussen and Dr. Russell Thomson were responsible for general advice regarding the study and editorial comments. Dr. Russell Thomson and Dr. Simon Wotherspoon provided statistical support related to the development of the methodology. Markus Juonala, Niina Pitkänen, Terho Lehtimäki, Jorma S.A. Viikari, collected the clinical data and biological samples used in this study and provided clinical insight as well as editorial comments. Olli T. Raitakari as the principal investigator of the YFS study provided an overall advisory and editorial role. All listed authors reviewed and approved the final version of manuscript.

The paper reported in Chapter 5

Body mass index trajectories associated with resolution of elevated adiposity in youth and incident adult obesity.

Marie-Jeanne Buscot as first author was responsible for the concept of the paper, data analysis, interpretation, writing all drafts and addressing co-authors' comments. Dr. Costan Magnussen and Dr. Russell Thomson were responsible for general advice regarding the study and editorial comments. Markus Juonala, Niina Pitkänen, Terho Lehtimäki, Jorma S.A. Viikari, collected the clinical data and biological samples used in this study and provided clinical insight as well as editorial comments. Eero Jokinen, Paivi Tossavainen, Tomi Laitinen provided editorial comments and were involved in data collection. Matthew Sabin and David Burgner edited the mansucript and helped identify the clinically relevant findings to target the paper to a clinical audience. Olli T. Raitakari as the principal

investigator of the YFS study provided an overall advisory and editorial role. All listed authors reviewed and approved the final version of manuscript.

The paper reported in Chapter 6

Qualitatively distinct child to adult body mass index (BMI) trajectories and adult cardiometabolic outcomes

Marie-Jeanne Buscot as first author was responsible for the concept of the paper, for the data cleaning, statistical analysis, drafting of the manuscript and coordination of the manuscript revisions. Dr. Costan Magnussen and Dr. Russell Thomson were responsible for general advice regarding the study and editorial comments. Markus Juonala, Niina Pitkänen, Terho Lehtimäki, Jorma S.A. Viikari, collected the clinical data and biological samples used in this study and provided clinical insight as well as editorial comments. Matthew Sabin and David Burgner provided critical revision of the manuscript. Olli T. Raitakari as the principal investigator of the YFS study provided an overall advisory and editorial role. All listed authors reviewed and approved the final version of manuscript.

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Date: ……07/11/2017…….....

Principal supervisor signature: …….

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LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following four original manuscripts. The manuscripts are ordered as they are cited in this thesis.

I The Combined Effect of Common Genetic Risk Variants on Circulating Lipoproteins Is Evident in Childhood: A Longitudinal Analysis of the Cardiovascular Risk in Young Finns Study. Buscot MJ, Magnussen CG, Juonala M, Pitkänen N, Lehtimäki T, et al. (2016). PLOS ONE 11(1): e0146081. doi:10.1371/journal.pone.0146081.

II Bayesian hierarchical piecewise regression models: A tool to detect trajectory divergence between groups in long-term observational studies. Buscot MJ, Wotherspoon SS, Magnussen CG, Juonala M, Sabin MA, Burgner DP, Lehtimäki T, Viikari JSA, Hutri-Kähönen N, Raitakari OT, Thomson RJ (2017). *BMC Methodology in Medical Research*. 17(86). DOI: 10.1186/s12874-017-0358-9.

III Body mass index trajectories associated with resolution of elevated youth BMI and incident adult obesity. Buscot MJ, Thomson RJ, Juonala M, Sabin MA, Burgner DP, Lehtimäki T, Hutri-Kähönen N, Viikari JSA, Jokinen E, Tossavainen P, Laitinen T, Raitakari OT, Magnussen CG. *Accepted for publication in Pediatrics (August 2017).*

IV Qualitatively distinct child to adult BMI trajectories and cardiometabolic outcomes in mid-adutlhood. Buscot MJ, Thomson RJ, Juonala M, Sabin MA, Burgner DP, Lehtimäki T, Hutri-Kähönen N, Viikari JSA, Raitakari OT, Magnussen CG. *Manuscript submitted to JAMA Pediatrics.*

ABBREVIATIONS LIST

AIC = Akaike Information Criteria

ANCOVA = Analysis of covariance

ANOVA = Analysis of variance

BHPR = Bayesian hierarchical piecewise regression

BIC = Bayesian information criteria

BMI = body mass index

CFI = Comparative fit index

CHD = coronary heart disease

CI = confidence interval or credible interval

cIMT= carotid intima media thickness

CP =change point

CVD = cardiovascular disease

DIC = deviance information criteria

GCM = Growth Curve Modeling

GLM = generalised linear model

GMM = Growth Mixture Modeling

GWAS = Genome wide association study

HDL-C = high-density lipoprotein cholesterol

HLM = hierarchical linear model

ICC = intra-class correlation coefficient

IGC = Individual Growth Curve

LCA =Latent Class Analysis

LDL-C = low-density lipoprotein cholesterol

LCGMM = latent class growth mixture modeling

LGCM = latent growth curve modeling

ML = maximum likelihood

MANOVA = multivariate analysis of variance

MAR = missing at random

MCMC = Markov chain Monte Carlo

MLM = multilevel model

NCEP = National Cholesterol Education Program

OR = odds ratio

REML = restricted maximum likelihood

RM-ANOVA = repeated-measure analysis of variance

RMSEA = root mean square error of approximation

RR = relative risk

SE = standard error

SEM = structural equation modeling

SD = standard deviation

SNP = single nucleotide Polymorphism

TC = total cholesterol

T2DM = type 2 diabetes mellitus

TG = triglycerides

TIC = time-invariant covariate

TLI = Tucker Lewis index

TVC = time-varying covariate

YFS =Cardiovascular Risk in Young Finns Study

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1. INTRODUCTION

Longitudinal data, collected on the same participants at multiple times during their lives or throughout an intervention, are common in epidemiological studies[1-4]. Longitudinally collected data enables the natural history of disease or progression in risk factors in a study sample to be characterised, and can be used to evaluate the effect of behavioural or pharmaceutical interventions on the outcome[5, 6]. When collecting these data, researchers are interested to determine, estimate, or capture the defining characteristics of change in health markers over time, and relate these to potential exposures or individual factors[7]. For conditions with a long latency period such as those that develop into clinically manifest diseases over decades, there is a growing interest in identifying the patterns of change in modifiable health markers that distinguish those who develop later diseases [8-10], and to identify the critical phases of change that may help inform prevention or intervention[11-17].

Growth curve, or trajectory, modeling is a universal term commonly used to refer to a number of similar contemporary methods to analyse longitudinal data[5, 18-22]. These methods are flexible in their application and overcome many of the limitations of traditional approaches to repeated measure data analysis[23, 24]. One of the strengths of growth curve modeling approaches is that they allow outcome trajectories to be modeled at the population and at the individual level simultaneously. This group of methods thus enables the explicit investigation of between-person differences in intra-individual change, as well as determinants of change over time[25-29].

Initially developed and used in the fields of psychology, education, and developmental research, growth curve modeling has developed considerably in the last 30 years and remains the object of ongoing development. Although complex, these methods have gained popularity across disciplines, and are increasingly easy to implement thanks to the development of powerful statistical software and ongoing advances in bioinformatics and computer technologies. However, the technical nature

of the published literature and inconsistencies in nomenclature used, make it challenging for many investigators, including epidemiologists, to understand the current state of the growth curve modeling literature, and how and when to apply the most appropriate techniques across different practical situations[5].

Another potential reason for the lack of up-take of trajectory models among epidemiologists is that applying these methods to longitudinal data from long-running observational studies comes with an additional set of specific technical challenges. These challenges include sporadic follow-up of participants over large and varied time-windows, that can span from childhood through to older age, and generally high rates of loss to follow-up or intermittent drop-out – all factors that can influence the choice and implementation of trajectory models. Many prospective studies are also conceived as multi-cohort studies or accelerated designs to span the age-range of interest in a shorter period of time than with a single cohort to make the study more cost effective[4, 22]. In growth curve modeling, joining repeated-measure data collected from multiple birth cohorts to model and compare longitudinal change in response across the ages spanned by the entire cohort is not straightforward. Another significant challenge is the potential non-linearity of the biological response of interest as a function of time or age. Many trajectory-modeling applications in the literature consider linear change over time[18, 19]. Although this may be a reasonable assumption for some biological processes when the time-window is relatively short, many variables are likely to behave non-linearly over time or across several developmental phases. Being able to model non-linear responses over time with only a few measures per participant is a difficult and often overlooked aspect of growth curve modeling in the literature, which complicates the application of these methods to long-term prospective cohort study data.

1.1 General and specific aims of the research

The general aim of this thesis is to examine growth-modeling methods for continuous longitudinal data with a special emphasis on how these techniques can be used to investigate pathways leading to deleterious cardiometabolic disease outcomes in adulthood.

The specific aims are to:

Aim 1. Identify, review and compile the different key issues and analytical strategies relative to the application of growth curve modeling to continuous response data arising from long-term observational studies with relatively few (<10) and sparse time points per study participant. The particular focus is on how these strategies can be implemented and used to analyse non-linear response profiles when individual follow-ups span several developmental periods across the life course (i.e. childhood, adolescence and adulthood) and when the study sample comprises several birth cohorts (i.e. accelerated longitudinal designs or multi-cohort studies).

Aim 2. Tailor and extend growth curve modeling theory to develop a "trajectory divergence" method, providing a new tool to help inform prevention and intervention strategies. Specifically, the "trajectory divergence" method was developed to identify the point in the life course at which participants who develop adverse health outcomes in later life begin to diverge in their non-linear trajectories of continuous modifiable risk factors, compared with those who remain healthy.

Aim 3. Apply the statistical methods reviewed in Aim 1 and developed in Aim 2 to model and analyse the longitudinal trajectories of a number of continuous measures of cardiovascular risk from the YFS study, an accelerated longitudinal prospective study that has collected cardiometabolic disease risk factor data in a large sample of participants from early childhood to mid-adulthood across eight waves of follow-up.

1.2 Importance of cardiometabolic disease

Although longitudinal statistical methods, and growth curve modeling in particular, were the primary motivation for this thesis, the questions of interest arising from the YFS drove the direction of the research. This section provides a brief overview of the importance of cardiometabolic disease that sets the scene for the formulation of the specific research questions examined by this thesis.

Cardiometabolic disease is a collective term referring to cardiovascular disease (CVD) as well as metabolic disorders such as the type 2 diabetes mellitus (T2DM)[30]. CVD is single biggest killer worldwide[30, 31]. More people die annually from CVDs than from any other cause. The World Health Organisation report that an estimated 17.5 million people died from CVDs in 2015, representing 31% of all global deaths. Although the general trend of death from CVD has been declining since the 1950s in developed countries, thanks to advances in pharmacological intervention and favourable changes in risk factor distribution, they are becoming an increased burden to low-and middle-income countries, with over three quarters of CVD deaths taking place in developing countries as of 2015[30]. Atherosclerosis, a condition in which plaque builds up inside the arteries is recognised as the main cause of CVD[32]. Although the major clinical complications of atherosclerosis do not typically present until middle or older age, the atherogenic process has been shown to begin in early life and to progress from an asymptomatic phase to a clinically manifest disease over decades[12, 33].

T2DM is a progressive chronic condition in which the body becomes resistant to the normal effects of insulin or gradually loses the capacity to produce enough insulin in the pancreas[34]. T2DM greatly increases the risk of heart disease and stroke, as the condition tends to cluster with other interrelated cardiometabolic risk factors including obesity, hypertension, and dyslipidemia[35, 36]. Adults with T2DM are two to

four times more likely to die from heart disease than adults without diabetes, and more than 80% people 65 years or older with diabetes die from some form of heart disease or stroke[37]. The American Heart Association considers diabetes to be one of the seven major controllable risk factors for cardiovascular disease[38].

There are a number of well-identified risk factors for cardiometabolic diseases. While some are not modifiable such as genetics, sex, and increasing age, many risk factors such as obesity, hypertension, dyslipidemia and smoking are amenable to lifestyle changes and therapeutic interventions. Most cardiometabolic diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, poor cardiorespiratory fitness and harmful use of alcohol using population-wide strategies [39-41]. In fact, the trend of decrease in CVD death since the 1950s has been largely attributed to intervention on these modifiable risk factors in adults.

In trying to determine the origins of cardiometabolic disease, there has also been an increased interest since the 1970s in studying the progression of these risk factors across the life course with the motivation to better understand who is at risk of developing clinical complications in later life and how or when to intervene to reduce the incidence of cardiovascular disease in the general population[42-46]. The rationale is that if children with one or more of the major modifiable risk factors are at an increased risk of developing clinically significant disease later in life, then controlling these cardiometabolic risk factors in early life may help reduce the incidence of clinical disease or cardiometabolic-related mortality later in life.

Among the modifiable risk factors for cardiometabolic disease, the role of excess adiposity (termed overweight and obesity and usually based on an individual's body mass index, derive from and individual's weight and height) in youth is one important risk factor that has been linked with incident cardiovascular events [47-51] and with early markers of cardiovascular disease (such as increased carotid intima media thickness

(cIMT) and increased arterial stiffness, which indicate early stage atherosclerosis) in young adulthood [33, 52].

Additionally, as with findings in adult populations, there is growing evidence that blood lipid and lipoprotein levels (e.g. cholesterol in the blood) measured early in life also play an important role in the development of cardiometabolic disease [46]. Prospective cohort studies have shown that lipoprotein levels track strongly from childhood and adolescence to adulthood [11, 53], so that children with high lipid and lipoprotein levels have a greater risk of having elevated adult levels than their peers with lower levels [50, 54, 55], although most of these children do not have adult levels that meet cut-points for intervention. There is also evidence that adverse lipoprotein levels in early life may induce arterial changes that contribute to adult atherosclerosis, thus contributing to CVD risk [56-58].

In the absence of carefully controlled studies, prospective longitudinal studies and accelerated cohort studies of cardiometabolic risk where participants are examined multiple times from childhood through adulthood are particularly useful to study risk factors influencing the development of adverse cardiovascular profiles and deleterious cardiometabolic outcomes across the life course, while addressing key limitations of cross-sectional studies. They provide a chance to investigate who is at highest risk of developing significant risk factors or early stage cardiometabolic disease, at what time in the life course, and factors associated with their development. It is thus crucial to have the right statistical tools to analyse data arising from this type of studies.

Devised to examine the childhood origin of adult CVD, the YFS is a large ongoing Finnish population-based prospective cohort of cardiovascular risk factors and their determinants. It is one of only few studies worldwide to have collected cardiometabolic risk factors data since childhood with follow-up measurements occurring through to adolescence, young and middle adulthood[59]. This thesis takes

advantage of the eight examination waves of data collected on the same YFS participants from childhood to adulthood over the course of 31 years. A number of cardiometabolic risk factors and health markers measured at these eight examinations were used to study the aims of this thesis. Detailed descriptions of the YFS, including study design, participant characteristics, and attrition analyses have been published previously[60-62]. Appendix 1.1 provides some background on the YFS study that is not covered in subsequent chapters.

1.3 Thesis structure

This thesis has been written as a series of original papers included in Chapters 3 to 6, which have been (or will be) submitted to journals and have been left in their entirety. These chapters each address the pivotal aims 2 and 3 of this thesis. When relevant, material included as supplementary only material in the corresponding manuscripts was integrated into the chapters.

Chapter 2, the literature review provides the background and foundation for the methodological component of the thesis and gives more detailed grounding than is incorporated in each paper. It reviews traditional person-based methods and special considerations when applying these methods to long-term prospective cohort data, as well as key aspects to be considered when deciding to implement them in the multilevel (MLM) or in the latent growth curve (LGCM) modeling framework. It discusses the implementation of growth models in the frequentist and Bayesian framework, with particular emphasis on analytical challenges that accompany the analysis of data from accelerated longitudinal study designs. Chapter 2 discusses model-building strategies and different parameterisations of growth curve models within the MLM framework to adequately represent the non-linearity of the continuous biological responses over time. It also introduces the piecewise parameterisation of the MLM growth model and key aspects of its estimation within the Bayesian framework, which are used and developed further in Chapter 4. Finally,

it presents the increasingly popular and often complementary alternative to individual-based growth models, also known as Latent Class or group-based approaches, which allow participants to be clustered according to their developmental progressions in the outcome over the life course and identify underlying trajectories that may not be identifiable when using classic growth models and *a-priori* classification rules of participants.

Chapter 3 addresses research aims 1 and 2 in the frequentist framework for 2442 YFS participants for whom genetic information was available for a total of 76 lipid-associated Single Nucleotide Polymorphisms (SNPs) reported in the literature. The primary aim was to quantify the contribution of a multigenic lipoprotein score to adverse levels of circulating lipoproteins, important risk factors for cardiovascular disease. A second question addressed whether multigenic predisposition to adult dyslipidaemia, having an at-risk lipoprotein profile, was modified by the magnitude of the change in body mass index (BMI) from childhood to adulthood, used as a proxy indicator for lifestyle trajectory. In this chapter, the clustering of participants arises due to individual characteristics, that is, belonging to a given genetic risk category. Multilevel Growth Curve models, parameterised with higher-order polynomial terms of age to represent the non-linear lipid trajectories within-participants, were used to investigate if the change in lipid over time was different in high-risk participants compared with participants classified as low-risk.

Chapter 4 formulates and develops a Growth curve modeling methodology based on Bayesian hierarchical piecewise regression (BHPR) to investigate trajectory divergence in continuous response profiles between two or more groups of participants in cohort studies and to determine when groups of interest begin to show different non-linear trajectories in continuous response data. To represent the curvilinear development of BMI between age 6 and 49 years, this method uses a two-piece linear–linear spline model as the functional form of the MLM growth curve model. The piecewise parameterisation of the growth curve model overcomes some

of the numerical and interpretation issues commonly encountered with higher order age-polynomials by comparing the growth rate between different periods of development that results in biologically meaningful coefficients. To test for between-group divergence in key aspects of the longitudinal response, the method introduces dummy variables that represent group membership as time-invariant predictors of model parameters at level-2, which shifts the marginal means of different growth parameters in one group compared with the other groups. Because the focus was to estimate the position of the change-point in each group and allow for between-person heterogeneity in the age at which each participant switches developmental phases (i.e. individual change-points), a hierarchical random change-point model in a fully Bayesian framework was implemented that introduced key aspects related to Bayesian model estimation, comparison, and validation. The application of this method is outlined by modeling individual BMI trajectories in a subset of 2540 YFS participants to investigate the mechanism of childhood to adulthood BMI trajectory divergence between participants who developed adult T2DM in adulthood, a major metabolic disease, compared with those who did not develop T2DM. In a simulation study, the age and confidence interval at divergence is estimated and compared to more traditional approaches.

Chapter 5 uses the BHPR approach developed in Chapter 4 and extends it to suit the estimation of a multiple-group growth model. Specifically, a slightly modified parameterisation of the conditional BHPR model was formulated to estimate and compare developmental BMI trajectories among four *a-priori* determined child to adult adiposity status groups in 2717 YFS participants. This four group-classification identified participants who either remained obese, resolved obesity, remained non-obese, or became obese based on their BMI status between two YFS follow-up examinations: one in childhood (baseline study) and the latest available individual BMI assessment in adulthood. Using this classification method, a large-scale multi-study previously found that participants who resolved adverse BMI levels between their child and adult assessments had similar cardiovascular risk levels to those who

were never obese. It was also found that obese adult participants were at increased cardiovascular risk, irrespective of their childhood adiposity status. Since some population samples in this multi-cohort study had only two waves of data collection, the BMI trajectories of participants within these four groups of clinical interest had not been investigated. Taking advantage of the eight measurement time-points in the YFS, the aim of Chapter 5 was thus to characterise and compare the average BMI trajectories from childhood to adulthood within these four adiposity groups to identify critical age windows for the development and resolution of adverse adiposity status in this large cohort. This is crucial to help understand the dynamics of pathological vs. normal BMI trajectories across developmental periods, which could help inform weight management interventions.

Chapter 6 investigates potential heterogeneity in BMI development in the YFS population from a life course perspective, asking whether distinct long-term BMI developmental patterns result in different cardiovascular risk in adulthood. Indeed, the clustering of participants into the four adiposity status groups considered in Chapter 5 was performed on the basis of only two time points only and the definition of adiposity cut-points in childhood and adulthood was done in a subjective manner. This approach ignored the potentially different dynamics of obesity resolution or onset within a given adiposity group. It was hypothesised that different BMI trajectory patterns exist within the YFS population, which could not be identified using the traditional person-based growth modeling approaches (such as the BHPR method), and that these underlying trajectories may be differently associated with adult CVD risk. Therefore, among a subset of 2631 YFS participants, latent class growth mixture modeling (LCGMM) was applied to: (1) identify subgroups who share similar trajectories in BMI from childhood though middle age; (2) provide a preliminary characterisation of these participants and BMI trajectories; and (3) determine the independent association of identified latent life course BMI trajectories with adulthood cardiovascular risk factors of T2DM, dyslipidaemia, hypertension

(high blood pressure) and preclinical atherosclerosis, a condition considered as the major underlying cause of cardiovascular disease.

Chapters 3 to 6 have been written as a journal articles, for which Buscot Marie-Jeanne is the first author and are presented in their entirety. Declaration of contribution of authors to these research articles in Chapters 3 to 6 can be viewed in in the statement of authorship. Additionally, a description of how each chapter fits with the specific aims of this thesis is provided at the beginning of each chapter, in preface sections.

1.3 Overview of the YFS Study

Since they examine different cardiometabolic risk factors or outcomes and address different research questions, thesis chapters 3, 4, 5 and 6 each include a different subset of YFS participants. For each study, inclusion criteria of participants are covered in detail in relevant chapters, along with the statistical methods specifically employed to address the different research questions and the identified aims of this thesis. This appendix aims at providing a general overview of the YFS study, the primary data source utilised to examine the aims of this thesis.

Study sample

The YFS study is an on-going five-centre epidemiologic study of atherosclerosis risk factors and precursors from childhood to adulthood. It was devised in the late 1970s as a collaborative effort between the departments of paediatrics or medicine of Helsinki, Kuopio, Oulu, Tampere, Turku university hospitals to assess biological and lifestyle factors underlying cardiovascular disease and their determinants in children and adolescents of various ages in different parts of Finland. It was then pursued over 31 years to study these risk factors and their determinants from childhood to adulthood in the Finn population.

The YFS study design and sampling procedure have been described in details previously, but we will provide a summary here. The first main cross-sectional (baseline) study was performed in 1980, where 3596 children aged 3 to 18 years, randomly chosen from the Finnish Social Insurance Institution's national population register from five regions in Finland, were assessed for many cardiovascular risk factors including anthropomorphic measures, blood samples, blood pressure, lifestyle information, They belonged to 6 birth cohorts each separated by 3 years (i.e. years of birth of baseline participants were 1977, 1974, 1971, 1968, 1965 and 1962). Thereafter, follow-up studies were conducted at intervals of three years until 1992. Three adult follow-ups of the sample were conducted 21, 27 and 31 years later respectively, in 2001, 2007 and 2011. At the latest adult follow-up in 2011, the 1999 participants seen in clinic (55 % of the original cohort) were aged 34, 37, 40, 46 and 49 years old. The study was conducted according to the guidelines of the Declaration of Helsinki, and local ethics committees approved the study protocols. Written informed consent was obtained from all participants in 2001 and their parents in 1980.

This thesis takes advantage of these 8 examination waves of data collected on the same participants from childhood to adulthood over the course of 31 years. A number of CVD risk factors and health markers measured at these 8 examinations were used to study the aims of this thesis.

Measures

Detailed descriptions of data collection procedures of the different follow-up clinics can be found elsewhere. At each examination, a questionnaire that collected data on socioeconomic status, living environment, health, physical activity, and parent and grand-parent health was mailed to the participants. Series of measurements were collected at the time of the physical examination, including anthropometry, blood pressure, blood samples, and ultrasound studies of the brachial and carotid arteries (in

2001, 2007 and 2011). Relevant to this thesis are the anthropometry measures and blood samples.

Blood collection and biochemistry

All serum lipid determinations were done on fasting samples in duplicate in the same laboratory. In 2001, serum cholesterol and triglyceride concentrations were determined enzymatically (Olympus System Reagent, Olympus Diagnostica, Hamburg, Germany) in a clinical chemistry analyser (AU400, Olympus Optical, Mishima, Japan). HDL cholesterol was analysed after precipitation of very low-density lipoprotein and LDL with dextrane sulphate 500 000. The concentration of LDL cholesterol was calculated using the Friedewald formula. Details of the methods in earlier studies have been previously published. Due to changes in determination methods and kits during study years, lipoprotein levels from 1980 were corrected to those in 2001 using correction factor equations (this information is detailed in Chapter 3 Methods). T2DM status was assessed at adult follow-ups in 2001, 2007 and 2011.

Participants were considered diabetic if they had a fasting plasma glucose level of 7 mmol per liter (126 mg per deciliter) or higher, reported the use of oral glucose-lowering medication or insulin but had not reported having type 1 diabetes, or had received a diagnosis of T2DM from a physician at any of their adult follow-up examinations.

Anthropometry

Height and weight measures were taken at all examinations using the following protocols. Height was measured using a wall-mounted Seca stadiometer with 0.5 cm accuracy, and weight was measured in light clothing without shoes with a digital Seca scale to the nearest 0.1 kg. Baseline and follow-up BMI measures were determined from weight and height measurements recorded at each clinic using the formulat $BMI = Height\ (m) / weight^2\ (kg^2)$.

2. LITERATURE REVIEW: GROWTH CURVE MODELS FOR LONG-TERM COHORT STUDIES

Longitudinal designs, with data collected on the same participants at multiple times during their lives or throughout an intervention, are increasingly popular in a variety of disciplines, including clinical and observational epidemiology[1]. They are very valuable either to characterise the natural history of disease or risk factors in a cohort or to evaluate the effect of behavioural or pharmaceutical interventions on the outcome over time[63]. Researchers are often interested in understanding, estimating, and capturing the defining characteristics of change processes or progression in outcome data, and relate these characteristics to potential predictors, such as exposure or risk factors. Growth curve or trajectory modeling, a catch-all term for a number of similar regression based techniques traditionally developed in psychology and developmental research, have gained popularity across disciplines thanks to the development of powerful statistical software[5, 64].

Growth Curve Modeling (GCM) allows outcome trajectories to be modeled simultaneously at the population and the individual level, to explore between-person differences in intra-individual change, as well as determinants of change [18, 19, 23, 24]. Because the framework of growth modeling bridges both Structural Equation Modeling (SEM) and Multilevel Modeling (MLM) traditions, many terms refer to broadly equivalent techniques, including Hierarchical Modeling of change (HLM) [65-67], Mixed Effect / Random Effect Modeling[68], and latent growth curve models (LGCM)[69-72]. Many statistical textbooks describe these different growth-modeling techniques, but most are highly mathematical and often outside the reach of most epidemiologists. Few focus specifically on the application of these methods to epidemiological data, and since each field has a preferred nomenclature, it makes it challenging for epidemiologists to understand the current state of the growth curve

modeling literature, as well as to identify what is the most appropriate technique in different practical situations. Although MLM and LGCM models have been widely adopted for over two decades in fields such as education, sociology and psychology, where they are routinely used to model growth and change in behavioural and cognitive processes, these approaches remain underused in observational epidemiology[5, 73-76]. 'Traditional' longitudinal data analysis methods tend to be employed, suggesting that the field has yet to realise the potential and possibilities of these methods. This is particularly unfortunate in light of the extensive and informative databases that have been collected over the past century through observational studies, to help understand conditions that have long latency periods before clinical manifestations, particularly chronic conditionas such as cardiovascular diseases and cancers. Additionally, the misuse of traditional approaches, if underlying assumptions are not met, can lead to inappropriate and inefficient analysis, producing inaccurate results, and simplistic or incorrect interpretation of findings.

The lack of popularity of trajectory modeling techniques among epidemiologists and population health investigators can also be partly explained by the set of specific technical challenges faced by researches when applying these methods to longitudinal data from long-running observational studies. This includes high rates of missing data, and multi-cohort data, both of which can influence the type of trajectory model to be used in the analyses[4, 22, 77, 78]. Another complication to the application of these models to cohort studies, is that across fields, many applications and examples of growth modeling in the literature consider and model linear change patterns in the response over time, because of their simplicity and interpretability. However, many biological, developmental processes, and empirical data are often more complex, and are not adequately characterised by linear rates of change over time [79-81]. This is particularly true in long-running observational or life course studies, where responses of interest may be collected on participants followed sporadically over relatively long time-windows, sometimes spanning from childhood through to older age. Although some biological continuous outcomes are expected to have specific non-linear

trajectories over time, in many situations there might not be any existing theories regarding the nature of the trajectory of the continuous outcome over time. Whether it is conceptualised as a SEM or a MLM, there are several ways in which the basic linear growth model has been developed and expanded in recent years to model complex non-linear relationships between the continuous response variable and the relevant time variable. However, correctly specifying the functional form for the within-person change over time (i.e. the form of the average relationship between age or time and the outcome) is crucial in the specification of the growth model and the choice of the non-linear functional form of change within-person is not straightforward.

In this chapter, an organised overview of different growth curve modeling techniques for analysing response data in epidemiology, including model specification, estimation, and validation, is provided. Specifically, the different conceptual paradigms, strengths, and limitations of a number of approaches to model potentially complex non-linear trajectories of continuous outcomes are highlighted. The specific challenges that arise in these methods when the longitudinal data comprises several birth-cohorts is also discussed, since many long-term prospective observational studies in epidemiology are designed as accelerated longitudinal designs, also known as multi-wave or multi-cohort panel studies. These studies typically include participants from multiple birth cohorts, which may be followed for a few years or sporadically over a few decades over the same study period. Accelerated longitudinal designs enable researchers to study individual change over a long interval of the life course by gathering data during a comparatively short interval of time, and provide opportunities to separate developmental effects from period and cohort [78]. However, these types of designs also confront researchers with a special set of inferential challenges and introduce significant complexity into statistical modeling.

In this chapter, the critical aspects of the different growth curve modeling approaches are highlighted, and situations in which some methods may reveal more appropriate than others are suggested. This background provides the methodological foundation and background of this thesis, and identifies critical technical aspects that pertain to using a range of growth curve modeling methods to analyse continuous data from long-running prospective cohort studies.

2.1 Introduction: Longitudinal data in epidemiology

In this section, the advantages and challenges of longitudinal panel studies are discussed, including situations where traditional methods, such as repeated measures and multivariate Analysis of Variance (ANOVA), can be used and where they are not appropriate for this type of data.

2.1.1 Longitudinal panel studies

Longitudinal assessments offer significant advantages over cross-sectional studies as they allow the separation of within- and between-subject variability and they are less prone to confounding[82]. **E**xperimental and observational epidemiologists are often interested in understanding and capturing change processes in continuous measures of health outcomes, and to estimate the defining characteristics of these changes among individuals [5, 83, 84]. This is useful to help identify adverse as well as beneficial factors that influence healthy and pathological change in health outcomes over time, or understand what factors may mitigate adverse change in these outcomes. To do so, many epidemiologic studies routinely collect repeated measures of continuous health outcomes on a number of participants, often stratified in *a-priori* groups.

Longitudinally collected data arise from many observational study designs (case-control, retrospective and prospective cohorts) as well as in experimental studies (randomised control trials or non-randomised control studies). In clinical trials,

subjects can be followed for a few days, weeks or months, to investigate how an intervention of interest affects specific health outcomes. While outcomes measures are often measured at least twice (i.e. classic 'pre-post' design), intermediate measures have become increasingly common, as medical researchers become interested in the change patterns themselves to try and understand pathways or trajectories that lead to disease or remission. In prospective observational studies, especially in the case of adult-onset disorders, such as heart disease, cancer, and diabetes, investigators tend to follow study participants sporadically over relatively long periods to determine how a number of individual characteristics, risk factors, their interactions, and normal aging may impact the onset and the progression of disease in the population[85]. In other cases, the outcome of interest may be a continuous risk factor variable (blood lipid level, blood pressure value). When looking at longitudinal data clustered in groups, investigators often want to be able to distinguish an overall group effect (time-average effect) from a time-dependent group effect. In these instances, the question of interest might be how average response profiles of people with or without certain characteristics (sex, ethnicity) or with different levels of covariates (BMI, waist circumference) might change over time, and whether their outcome trajectories follow the same pattern or diverge as they age [63, 86]. The overarching aim in many longitudinal studies, is to be able to detect a significant difference between two (or more) groups of sequential observations, ascertaining the validity of the categorical "grouping" as a predictor of the response of interest [6].

One of the reasons for the popularity of longitudinal cohort studies is that despite remaining observational in essence, they are more useful for inferring causal relationships than cross-sectional studies which typically provide a snapshot of disease and risk factor distribution over a fixed period of time [2]. The advantages of longitudinal designs in epidemiology are numerous: they allow the recording of incident events, the prospective ascertainment of exposure, the measurement of individual change in outcomes and the separation of time effects (e.g. cohort, period,

age), and allow to control for cohort effects[1]. They also allow the researcher to consider the effect of aging and provide an opportunity to disentangle its interaction with specific health factors when analysing health and disease trajectories [7]. Indeed, temporal variations in the incidence of rare, chronic or degenerative diseases have become a subject of great interest to public health epidemiologists who look for recognisable patterns and associations with putative causal factors in order to gain etiological clues and possibly predict the incidence of disease in population groups. The longitudinal information is particularly valuable for traits that develop over time or have a variable age of onset. Prospective cohort designs are particularly suitable to study chronic diseases such as cardiovascular diseases, where large numbers of people are followed up for long periods before sufficient cases manifest, and where there is an expected long induction period between exposure and the manifestation of disease.

Many longitudinal study samples related to cardiovascular and cardiometabolic disease, collect anthropomorphic measures, biochemical markers of cardiometabolic disease, and behavioural information on the same participants for several decades, sometimes spanning from childhood to adulthood. These longitudinal or panel data, repeatedly observed or measured on a temporal basis of time or age, provide the foundation of the analysis of processes, which evolve over time, often referred to as growth or trajectory models. The statistical approaches to examine the role of growth trajectories in the developmental origin of health and disease have attracted considerable attention among medical and epidemiological researchers over the past three decades [28]. With repeated measure data, the researcher's interest often lies in describing a number of characteristics of the change process, including the within-person change, average change, between-persons' differences in change, and determinants (or predictors) of change [67, 87]. Moreover, in many research areas, individual subjects' trajectories themselves are increasingly considered promising biomarkers for early stage diagnosis, tracking of disease progression and monitoring of potential treatments.

In summary, longitudinally collected data offer numerous benefits to many branches, however the analyses of data arising from such designs pose a number of technical challenges. The critical aspect of data obtained from longitudinal studies is the inherent within-subject correlation that arises due to multiple measurements made on a single subject (or other experimental unit). It is necessary to allow for this correlation since it reflects the heterogeneity in the response at the population level that leads to specific deviations from the overall temporal response pattern. In additional to subject-level characteristics, the measurement errors in longitudinal data often exhibit temporal dependence (i.e. autocorrelated residuals) and heteroscedasticity [1, 63, 88].

2.1.2 Traditional methods for longitudinal data analysis

Traditional longitudinal methods for the analyses of repeated measure data include the (univariate or within-subject) repeated measures analysis of variance (RM-ANOVA), or the multivariate analysis of variance (MANOVA, including analysis of covariance (ANCOVA)). Both methods belong to the GLM (generalised linear model) procedure and handle correlated residuals that may arise in longitudinal data, but do not allow one to explicitly model the change at the individual level, which is treated as error variance[87]. In these approaches, time is treated as a categorical factor. The RM-ANOVA is the simplest method but also the most restrictive and is most suited for well balanced longitudinal designs with the same, relatively few and usually evenly spaced sampling occasions in time for each subject with no missing values and only accommodates time-invariant covariates [63, 84]. The RM-ANOVA is an omnibus statistical procedure that tests the null hypothesis that the means of the response are equal[1]. If the null hypothesis for between/within subject effect interaction is rejected, then the between-group differences vary across time. However, post-hoc group comparisons are required to investigate where the between-group differences occur. The RM-ANOVA rely on the assumption of sphericity of the covariance structure, of which compound symmetry is a particular form[1]. To provide

valid inference, the RM-ANOVA therefore requires equal variances at each time point and equal covariance between any time point, or it can lead to incorrect decision if these assumptions are violated [5, 63, 87]. Therefore, this technique is only appropriate if the investigators have a reason to believe that the correlations between all pairs of repeated measures are identical, which is rarely the case in longitudinal studies [5, 63]. Some correction factors such as the Greenhouse-Geisser, lower bound correction, and Huynh-Feldt methods were developed to adjust the degrees of freedom of the test so that the RM-ANOVA can be used when the sphericity assumption is not met [89 90] , but a well-documented drawback of these adjustment procedures is that they tend to be overly conservative and to incorrectly reject the null hypothesis.

The MAN(C)OVA (or multivariate ANOVA) approach transforms the repeated measures into adjacent differences, so that the null hypothesis becomes that the differences in pairs of means are equal to zero[64, 87, 91]. It is more flexible than the RM-ANOVA in that the assumption of sphericity is not required and polynomial contrasts or trends can be implemented to compare differences among time periods [2, 87], but there must be no interaction between the covariate and the independent variable (also called homogeneity of regression slopes). However, both RM-ANOVA and MANOVA models require complete data and fixed measurement occasions for all study participants, and both approaches conceptualise change as incremental rather than a continuous developmental process that unfolds over time [23, 24]. Guidance and further considerations on multiple testing issues and multiple comparisons among sets of repeated measures in RM-ANOVA have been well defined [64, 92 2, 93-95].

2.2 Person-based approaches: Linear Growth Curve Models

There are two approaches to modeling linear growth curves, MLM models and latent LGCM models that use the SEM framework. In Section 2.2.1, the differences between these two approaches are outlined. Section 2.2.2 outlines the formation of the linear growth curve model, section 2.2.3 describes the treatment of the time

variable, and section 2.2.4 describes how to formulate the hierarchical structure of the model.

2.2.1 Introduction to person-based Growth Curve Models

Growth curve modeling as provided a more flexible set of tools to model growth or change in outcome variables over time. This class for longitudinal analysis methods allows summarizing a set of within-subject time-ordered response variables with only a few parameters. Compared to the more traditional approaches described above, GCM permit one to describe and test hypotheses about inter-individual differences in intra-individual pattern of change, as well as predictors of these differences in the response trajectories over time [18, 19, 23, 67, 96]. Individual-based approaches to growth curve analysis examine the specific trajectory of the response variable for each participant and estimates informative characteristics of the growth pattern. Additionally, GCM allows one to further link observed change with specific exposures [97] by identifying covariates or mediating variables that might influence the patterns of response over time, in order to explain some of the between-participant variability in the response profiles (i.e. the determinant of change)[1, 98].These methods have become a popular framework in many disciplines, in particular in psychology, social sciences and education, due to their ability in helping researchers understand why and how individuals follow different developmental paths [25]. In medical research, GCM is an invaluable tool to understand the natural history of health outcomes, as well as risk factors trajectories [28, 97, 99].

Broadly, all person-based GCM approaches follow the same main steps: after selecting an appropriate function for age (or other time relevant variable) in order to model the average relationship between time and the longitudinal outcome, individual deviations from the intercept and age coefficient(s) are computed to capture each individual's deviation from the average trajectory. These individual summaries of the outcome or risk factor trajectory are often of interest and can be extracted and related

to later outcome using a two-step process [100] [101-103], or in a single step process in multivariate models [104, 105] using person-based GCM models. For example, to estimate the effects of change in weight at different life stages on health status in later life, a two-stage approach may be adopted. This consists of estimating changes in body size or growth velocity in different growth phases during the life course; then regressing health outcomes in later life on these estimated growth variables. Alternatively, growth trajectories as well as body size and their effects on later health outcomes can be estimated in the same model.

In contrast to RM-AN(C)OVA and MANOVA, GCM treats change as a continuous process that occurs between time points [24]. They are more flexible, and can accommodate the specificities of data arising from long-term prospective observational cohort data, such as those with partially missing data (e.g. intermittent loss to follow up or study drop-out), unequally spaced time points (unbalanced designs), non-normally distributed outcome measures, complex non-linear or compound-shaped trajectories, as well as the inclusion of time-varying covariates [63, 106, 107]. By convention, growth modeling assumes that the data is drawn from a single underlying population, with all subjects assumed to have developmental curves of the same functional form [24]. As mentioned previously, parameters describing individual curves may differ between-persons, so that growth curve analyses can be used to contrast the change process between multiple sub-populations of interest within the data sample [18, 19].

Compared to traditional approaches to repeated measure analyses, there are few data restrictions for GCM analysis. To reliably estimate a growth model, the number of individuals and the number of repeated measured per individual are important as they influence statistical power. Sample sizes of at least 60 participants at the highest level are typically preferred for hierarchical growth curve modeling using maximum likelihood (ML) techniques, but samples as small as 20 might be sufficient if the interest lies in the average growth parameters and not the estimate of the variance

components [99]. A recent simulation study showed that a small sample size of 50 or less at level-two (i.e. individual levels) leads to biased estimates of the second-level standard errors. In all of the other simulated conditions, estimates of the regression coefficients, variance components, and standard errors were unbiased and accurate [108]. There are many rules of thumb on the required sample size for MLM models (e.g. 15 units per cluster[22], 30 cluster/30 units per cluster rule [109], and the 50 clusters/20 units per cluster rule for detecting cross-level interaction effect [99]). However, none of these rules can provide an accurate estimation of the sample size required, given the power and target effect size [110]. Generally, a large number of higher-level units (participants in the case of observational studies) can increase the power for detecting the effects of higher level predictors and the cross-level interaction effects between within- and between-individual predictors [110].

Although it is possible to apply growth models to two-wave data, the traditional pre-post design is not optimal, especially if one intends to model individual heterogeneity in growth, since it is not possible to separate error variance from individual heterogeneity in change with only two time points [23, 24]. Furthermore, the existence of a functional form of change (time trend) more complex than a straight line cannot be identified with only two data points. In general, the simplest possible growth model requires at least three waves of data, but more reliable estimates for individual growth models can be obtained with a relative large number of measurements waves (e.g. 8 or more) [108, 110].

In GCM approaches, repeated measure data can be partially missing for a portion of the individuals if it is considered MAR (missing at random) [97, 107]. MAR is true when the probability of an observation being missing is related to other variables for that individual, but does not depend on the true value of the missing observation [111]. Under the MAR assumption, all individuals with at least one observation can contribute to the growth curve model. This is particularly important for longitudinal studies designed as accelerated or sequential cohort designs, where study attrition or

drop-out is frequent, and where subjects with a few or sparse observations across the study period will still contribute information to the analysis. An increased number of measurements usually positively affects the statistical power and reliability of the assessment of individual change [67]. In the case of cohort sequential designs, where a number of birth cohorts are followed over the same period of time to accumulate the age range of interest for a given longitudinal outcome, power is most influenced by the total life-span not the number of measures per birth cohort [4]. However, the number of repeated-measurements per individual, as well as the number of individuals per cohort or per age needs to be considered. The greater the number of cohorts, the longer the timespan in the trajectory analysis, but the risk of cohort differences in the change process becomes greater. Compared to traditional analytic approaches (e.g., RM-ANOVA) for the analysis of repeated measure data from between-group designs, growth models were shown to have greater statistical power at detecting a difference in linear slopes (i.e., time by group interaction) in small to moderate sample sizes [112].

Because different disciplines refer to broadly similar person-based GCM methods using different terminology (e.g., MLM[23, 113], individual growth curve analysis (IGC)[18, 19, 75], HLM[22, 67], mixed or random-effect models of change[84, 114, 115], random coefficient models[116] latent trajectory analysis[96, 97, 107], or [117, 118]), epidemiologists may find it challenging to understand the current state of the growth curve modeling literature, as well as identify what is the most appropriate approach to growth curve analyses in different situations. There are two approaches to traditional GCM models for longitudinal data: the MLM regression framework and the LGCM framework, which is a special case of SEM for longitudinal analysis [5, 28, 96, 117, 119]. Although they have different underlying assumptions, both approaches are closely connected and often numerically identical [97, 120] when it comes to analysing growth curves. Both LGCM and MLM growth models can accommodate a range of non-linear shapes for the outcome over time. In general, these models are fit using maximum likelihood (ML) or restricted maximum likelihood (REML) which can provide more accurate

results especially if the number of higher level units (e.g. often study participants) is small[67, 99]. Full ML estimation allows comparison of the goodness of fits of both fixed and random parts between nested models, whereas REML only compares the goodness of fit of the random part between nested models. REML estimation has been recommended by several authors to select the appropriate variance covariance matrix for the random effects [110, 121, 122]. Bayesian estimation of growth models is also possible in both MLM and LGCM frameworks, by addition of prior distribution assumptions on the parameters of the models, and by using combinations of Gibbs, Metropolis-hasting and reversible jumps Markov chain Monte Carlo (MCMC) sampling methods to generate posterior probability distribution for parameters [123]. Bayesian inference for hierarchical or multilevel models has been detailed by Gelman et al.[124] and Congdon[125]. Bayesian estimation is sometimes preferable to the traditional ML if the sample size is small, because it is less dependent on asymptotic assumptions [124]. As is shown in the next sections, some complex growth curve models may also be easier to estimate, or are only estimable, in the Bayesian context.

Because they differ in several analytical aspects, LGCM and MLM models require different software for estimation [97], and they differ in the type of criteria used for the assessment of model fit and approaches to compare multiple growth models. In the MLM framework, there is no stand-alone measure of overall model fit; model adequacy is assessed using comparison of potential candidate models. If models are nested, it is done using likelihood ratio tests, which compare models' deviances and the number of degrees of freedom based on the Chi-square difference statistics [67, 115]. If they are not nested, growth models can be compared using Akaike or Bayesian information criteria indices [126]. Pinheiro and Bates [114, 127] and Zuur et al. [121, 128] provide useful examples on how to compare competing growth curve models in the frequentist framework. On the other hand, the LGCM framework offers a number of indices for assessing model fit (i.e. root mean square error of approximation (RMSEA), Comparative fit index (CFI) and Tucker Lewis Index (TLI), among others). For a detailed overview on model fit and comparison in LGCM and MLM,

we refer to Hox et al. [99]. Applied epidemiologists can be confused about the differences and similarities between these two person-based growth modeling approaches. In the following section, the basic linear growth model is reviewed, and a number of selected analytical aspects that should be considered in the decision to apply one or more of these approaches is provided. For more thorough and technical descriptions of the MLM approach to growth curve modeling, we refer to [18, 19, 23, 24, 67, 116] and to [71, 107, 117, 120, 129] for LGCM modeling.

2.2.2 Linear Growth Curve Model

The simplest growth curve model is often a linear growth model, and like many other growth curves models, it can be estimated both as a latent growth curve model (LGCM using SEM) and as a HLM/ or MLM. Both one-stage and two-stage approaches can use the MLM or LGCM methods to model the growth trajectories and relate them to a later health outcome. This section will predominantly consider the two-level MLM/HLM model where the level-1 model represents the individual response trajectory over time (observation level) and the level-2 model considers changes in trajectory across subjects (between-person level). In this model, change or "growth" in the outcome variable is modeled simultaneously at the individual level and at the between individual level [130]. To illustrate the equivalence and differences of corresponding growth curve models formulated as LGCM in the SEM framework, LGCM model notation is used when necessary.

Unconditional model

The unconditional linear growth curve model is the model with no predictors or covariates at level-2. In multilevel (or hierarchical) notation, the level-1 part of the linear unconditional growth model corresponds to the repeated measures nested within-individual[22, 67]. It can be written as:

$$\text{Level 1}: \; y_{it} = int_i + slope_i . time_{it} + \epsilon_{it} \qquad (2.1)$$

where for time point *t* and participant *i*: y_{it} are the repeated measures of outcome y for participant *i (i=1,2,..,N)* , $time_{it}$ are the time related variables at the measuring occasion *t* (t=1,2,..T). Individual intercepts int_i and slopes $slope_i$ are the random coefficients. int_i represents each participant's predicted score when *t=0* (or any other time point that was used for centering the time variable); $slope_i$ represents the linear growth rate, which is the participant's predicted amount of change in y for a one unit change in *t* (or *t/k,* if a constant *k* was used to scale the time predictor to facilitate interpretation of the slope). The level-1 time-specific residuals ϵ_{it}, also known as error variance or occasion-level residuals, are often assumed to be distributed as $N(0, \sigma_e^2)$. They represent the measurement error and have constant variance although the model can be extended to incorporate more complex variance structure at the occasion level[75, 131] (see in section 2.3.2). Level 2-sub models can be written as:

Level 2 model: (2.2)

$$int_i = \beta_{00} + v_{0i}$$
$$slope_i = \beta_{10} + v_{1i}$$

where β_{00} and β_{10} are the mean intercept and mean linear slope in the population (i.e. population expectation for the fixed effects), and v_{0i} and v_{1i} are the level-2-random effects that allow the individual intercepts and growth rates to vary around the common trajectory specified by the population parameters. These level-2 random effects are in the most general case multivariate normal distributed as:

$$\begin{pmatrix} v_{0i} \\ v_{1i} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_{v0}^2 & \sigma_{v01} \\ \sigma_{v01} & \sigma_{v1}^2 \end{pmatrix}\right] \qquad (2.3)$$

where σ_{v0}^2 can be interpreted as the between-participant variability in the intercept, σ_{v1}^2 , the variability in the rate of change and where the covariance between random effects σ_{v01} represent the degree of correlations between individual's slopes and intercepts.

Equivalently, the linear curve growth model where each subject have their own linear regression curve with intercept and slopes varying randomly by subject, can be written as a LGCM 'measurement model' using the SEM notation as follows:

$$y_{it} = int_i.\lambda_{int} + slope_i.\lambda_{slope} + \epsilon_{it} \qquad (2.4)$$

In the LGCM formulation, int_i and $slope_i$ in 1.2 are referred to as latent variables, and time is introduced in the model via factor loadings λ_{int} and λ_{slope}. By convention for int_i, λ_{int} are all set to 1, and factor loadings λ_{slope} for $slope_i$ are set to the values of time *t* at each different measuring occasion, as in the MLM notation (i.e. [0,1,2,3] for a 4 time points design). Similarly to the MLM linear growth model, the latent variables have means (μ_{int} and μ_{slope}) and variances (σ^2_{int} and σ^2_{slope}) describing the population trajectory in the outcome and heterogeneity around the population curve.

Conditional model

In both the LGCM and MLM frameworks, it is often of interest to extend the linear 'level-1' or 'measurement' model by including time-varying predictors (TVC) and subject-level predictor (or time-invariant predictors (TIC)) of between-person heterogeneity in growth parameters at level 2 (also known as 'structural model' in the LGCM formulation). This allows testing whether certain groups of participants start with different response levels or change in a different way over time. Although all participants are assumed to have the same average trajectory over time (i.e. functional form of change), it is thus possible to examine how certain a priori groups deviate from the average population trajectory.

In the MLM framework this is sometimes referred to as the 'conditional' growth model since the individually varying growth parameters (i.e. intercept and slopes) are now conditioned on other predictors variables and not only on the time predictor. In the MLM notation, this is done by expanding the 2.1 and 2.2 equations as follows:

Level-1: (2.5)

$$Y_{it} = int_i + slope_i.time_{it} + \beta_t.TVC_{it} + \varepsilon_{it}$$

Level-2: (2.6)

$$int_i = \beta_{00} + \beta_{01}.TIC_i + v_{0i}$$
$$slope_i = \beta_{10} + \beta_{11}.TIC_i + v_{1i}$$

Where β_{01} is the mean difference in the intercept, β_{11}, is the mean difference in the linear slope, each respectively associated with the participant characteristic TIC; and where β_t accounts for the error variance within individual that is associated with the time varying predictor, TVC. The TICs thus directly predict the growth parameters, and the TVCs predict the repeated measures while controlling for the influence of the growth parameters [97]. If the subject level characteristic is a categorical variable (such as sex or binary coded dummy variable representing a grouping of interest), the categorical variable is used to shift the marginal mean of the growth parameters in one group to model difference in trajectories between clusters of individuals [97]. It is possible to build a more complex level-1 model with interaction between the relevant time predictor and the TVCs to test if the effect of the TVCs is constant over time (see [132]). Equations 2.1 and 2.2 show the inclusion of single TICs and TVCs covariates into the model, but multiple predictors can be included in the same way [22, 67]. Because of their quasi equivalence, we refer the reader to Hox and Stoel 2005 [99] for an exact mathematical representation of how TIC and TVC are incorporated into the basic linear LGCM model.

In the model presented in 2.1 and 2.5 we considered normally distributed model residuals, but it is important to note that growth curves model are not restricted to normal continuous outcome data. Growth models continue to be actively developed to address further restrictions imposed by empirically collected data, such as non-

normality of the response and multivariate responses. Through the use of link functions, linear mixed models can also be extended to allow for correlated response variables that have error distribution models other than a normal distribution (i.e. Poisson, binomial, exponential). Generalised mixed model and latent growth models for binary and ordinal outcomes are the subject of much ongoing research in the MLM and LGCM framework [3, 133-136].

2.2.3 Treatment of time in Growth Curve Models

The equations 1.1 and 1.2 presented for the basic linear growth model above, show that although they model the response within-person in the same way, the LGCM and MLM growth models differ fundamentally in the way the relevant 'time' variable enters the growth model, which has certain consequences for the analyses. In the MLM framework, time is incorporated as a fixed predictor variable (i.e. variable "t" in 1.1), while in SEMs time is introduced through parameters called factor loadings ($\lambda_{int}$and λ_{slope}), which are constrained to represent time in LGCM models [96]. As a consequence, MLM can be seen as a univariate approach, where time points are treated as different observations of the same 'time' variable, while LGCM can be seen as a multivariate approach where each time point is considered a separate variable[99]. This makes growth curves from LGCM generally best suited to analyse fixed occasion designs (i.e. subjects were assessed at the same follow-up clinics). In MLM, it is by design simple to include subjects chronological ages at each time point (i.e. age_{ij}) rather than measurement occasions themselves (i.e. $time_{ij}$) as the relevant time-variable. MLM growth models also deal naturally with incomplete data that may arise due to panel attrition or incidental dropout (under the MAR assumption [66]). It is thus generally best to use MLM if subjects were seen at varying occasions, if they were seen a different number of times, and if groups are of unequal sizes.

As mentioned previously, prospective observational cohort studies often include participants from multiple birth cohorts, which may be followed for a few years or sporadically over a few decades for the same study duration, so that the different cohorts remain age-heterogeneous at the end of data collection [4, 22, 78].
These accelerated, or "sequential-cohort" longitudinal designs, where the growth curve is estimated on a combination of cross-sectional and longitudinal data, are increasingly popular in epidemiology and other disciplines to study age-outcome trajectories over a broad age span during a study of short duration[28] [22]. In these cases, participants contribute data at different occasions and different numbers of occasions, with some overlap in their measuring occasions, so that a sample developmental growth curve spanning a longer period can be constructed using a number of groups that contribute different parts of the overall curve. Because the observable age-trajectories of different cohorts initiate and end at different ages, each cohort contributes to different and, to some degree, overlapping segments of cohort member's life course. Since response trajectories might differ across birth cohorts, (especially if the sample contains a mixture of generations) [65, 137, 138], it is necessary to take into account possible cohort effects When modeling the change in the outcome for the entire length of time spanned by the ages of the participants in all cohorts, The term 'cohort effect' in epidemiology, sometimes referred to as *birth* or *age* cohort effect, refers to the systematic difference in response trajectories between people born at different times[4].

To draw valid inference regarding growth parameters from accelerated longitudinal or cohort-sequential designs requires no cohort difference among the cohort-specific trajectories [139]. Indeed, the existence of age-by-cohort interaction effects may be due to demographic differences or to the effect of intervening variables that may lead to cohort differences in the age-outcome association [22, 65, 140]. Using data pooled from multiple cohorts may be inadequate to recover information concerning the entire longitudinal curve, and may yield distorted inference concerning the age-related

change. There are a number of strategies to deal with potential between-cohort heterogeneity in individual trajectories in the growth modeling framework [4].

In the MLM framework, early guidance on how to accommodate such accelerated designs in growth curve models was shown with data coming from 2 cohorts [65]. In a more recent paper, Miyazaki and Raudenbush [78] refer to this problem as "the test of convergence", that is, if the trajectories do not vary by birth cohort, the multiple-cohort design is thus considered "convergent'. In MLM models, testing for convergence in trajectories is usually done using dummy variables that represent cohort membership to augment the second-level model. This is sometimes referred to as the "fixed-effect" approach to cohort effect modeling. This consists of modeling cohort effect by including cohort interactions with each trajectory parameter of interest (i.e. intercept, linear change, quadratic change)[19, 23, 67]. The significance of age-by cohort interactions can then be tested using a Likelihood Ratio Test (LRT), in which the full model that includes dummy variables allowing for trajectories to differ by cohort is compared to a reduced model without the dummy variable, which posits a common trajectory model across all multiple cohorts [65, 78]. Another strategy is to adjust for any potential birth cohort effect by including centered "year of birth" or "age at first visit" of study participants as a level-2 covariate for all trajectory patameters[141]. This strategy considers that the fixed growth parameters vary linearly with year of birth, which is a simpler alternative than allowing for a completely different time trend in the response for each birth cohort. This approach results in more parsimonious models, and tends to be preferred when it is computationally difficult to estimate cohort-specific trajectories [4, 78].

Sometimes, trajectory models consider parallel longitudinal linear trends for the different cohorts but shifted by the intercept term (i.e. the cohort effect is only considered on the initial response level). If the curves for different cohorts are similar in regions of overlap, it may not be of concern that hypothesis tests indicate a significant difference in fixed effects parameters. This situation is likely to arise if the

regions of overlap are small, so that the information on the age range covered by a given birth cohort comes largely from that cohort [4]. As explained in Galbraith et al. 2014 [4], in this situation it is possible to derive fixed effects tests for differences between cohorts by integrating the absolute value of the difference between the estimated curves over the age range of overlap and to obtain standard errors via the delta method. Another, less popular, method that allows modeling between-cohorts differences in response trajectories is to add a third level to the MLM/ hierarchical model by including cohort-specific random effects. Appendix 2.3 illustrates how to model these fixed or random cohort effects on polynomial MLM models in practice in `R`, using the `nlme()`and `lme4()` functions.

Recent versions of SEM software (i.e. `Mx` and `Mplus`) now also allow to estimate LGCM models for designs with irregular time points, by modeling all existing occasions and viewing the varying occasions as missing data [21]. In the LGCM context, testing for cohort effects is conceptualised as a " multiple-group" approach, where cohorts represent a different pattern of "missingness" [140] and adjacent segments of limited longitudinal data from different age cohorts are linked together to determine the existence of a common developmental trend, or growth curve [72]. Several SEM indices can be used to assess "convergence" of trajectories from multiple cohorts in LGCM models [139] . Thus, when the number of cohorts is limited, and the maximum number of possible occasions remains limited across the study sample as a whole, SEM can now be used to fit LGCM models with sequential-cohort data [142,143 ,140]. However, it remains easier to test and adjust for cohort effects in the MLM framework since it can easily deal with attrition producing data missing at random. In SEM, each subpopulation in the multiple-group approach has to have the same data collection pattern. These models tend not to reach convergence as the number of time points increases and the number of missing-data patterns becomes large[78].

Many longitudinal data sets in epidemiology can present truly unique, individually varying time points, particularly if the time is measured precisely (i.e. age in days or months rather than years) or if data collection schedules are truly individual-specific. MLM remains the preferred framework for growth curve modeling in this situation, although some recent extensions in LGCM software and theory have been developed to handle this type of data (e.g. `Mplus`, `Mx` and `Open Mx`). Sterba 2014 [70] recently published a paper that illustrates how to use linear and non-linear LGCM models for data with subject-specific measurement schedules. However, this novel method is complex and due to convergence issues, it remains a challenge to estimate LGCM models with widely varying numbers of measuring occasions [70].

To summarize, the identification of the relevant time-metric is crucial as it impacts the choice of the approach to growth curve analysis, and it ultimately depends on the research question. In literature relevant to cardiometabolic research, when the interest lies in describing change patterns in cardiometabolic-related outcomes as a function of subjects ages, applications often use MLM growth curve models [74, 144-146]. This is the case of Heo et al [130], who used MLM models to develop BMI growth curves in overweight and obese adults. If study participants ages are not rounded up by year, but expressed in refined time scales such as days or months, it is likely that there will be a lot of 'unique' ages in the data and again, MLM will easily handle this situation. When the focus is on analysing the trend of an outcome over secular time, while controlling for subjects ages at first measurement, investigators tend to fit growth models as LGCM, within the SEM framework. For example, in a recent paper, Akbarzadeh et al[147], used LGCM to evaluate the trajectories of change in three anthropometric measurements within and between families seen at four measuring occasions separated by three years. The aim was to obtain an idea of the trend in obesity across 12 years, so although participants had various ages at the first wave (3 to 89 years), age was not the relevant time variable in the analyses (although the growth curve methods allowed to estimate the effects of age and sex on the growth rate of obesity indices). With the linear trajectory model (LCGM or MLM), with a

linear slope and no asymptotic constraints, individual trajectories of the outcome will project towards infinity if projected onwards. One should be cautious that even if appropriate to describe the change in outcome over a specific period (e.g. between ages 2 and 10 years), the model is not likely appropriate to make estimates outside of the study period.

2.2.4 Hierarchical data structure

The second important aspect to consider when choosing a growth curve modeling approach for a particular data set is the hierarchical structure of the observed outcome. In epidemiology repeated measures can arise from subjects sampled from a number of doctors, hospitals, or regions. In this instance, growth curve models must acknowledge the 3-level structure to produce unbiased results, because observations made by the same observer will be more similar than observations made by another observer. Defining 'clinician', as an extra level of nesting, allows to the results to be generalised to all clinicians (or a 'population' of doctors), so that inference is not restricted to the specific 'doctors' who made the observations in the study sample. Although confusion may arise with level identification in complex study designs, in general, "levels" are different to subject-level grouping variables (such as sex, ethnicity), in that they can be can be viewed as a random sample of a population of units. The MLM growth model expands naturally to higher levels of nesting (e.g. repeated measures nested within participant, nested in turn within clinicians, nested within hospital or region). Accounting for heterogeneity in the outcome trajectories due to nesting at higher level is more limited and technically difficult for growth models fit as LGCM. Traditional SEM software handle conventional two-levels models with repeated measures within individual at level-1 and predictors of between-subjects heterogeneity in trajectory at level-2 (`Mplus`, `Lisrel`) [148], but specific extensions of the method are required to estimate more general hierarchical structures via SEM, and the procedure is far more complex than MLM [149],[148]. MLM growth curve models remained preferred if there are more than 3 levels of nesting in

the longitudinal data [99]. For example, Briollais et al [150] used a MLM approach to model the longitudinal systolic blood pressure pedigree data arising from the Framingham Heart Study. In this application, individual repeated measurements (Level-1) where nested within individuals (Level-2), in turn clustered within genetic pedigrees (Level-3), which was a potential source of heterogeneity in the development of the systolic blood pressure profiles over age.

Effect of time-varying covariates and alternative variance-covariance structures

Although the linear growth model presented in both frameworks (equations 1.1. and 1.2) considered equal variances of the residuals at all measuring occasions and a unique effect of a TVC on the outcome across time points, these can be restrictive assumptions in many practical situations. For example, if the longitudinal outcome is blood pressure measured across the life course, and BMI is the time-varying predictor of interest, the researcher might be interested to test whether the effect of BMI on time-varying blood pressure is constant, or if it gets stronger or weaker as the subject ages. Because each occasion and TVC is considered a separate variable in LGCM, it is easy and often default to estimate all variances and covariances, as well as to estimate the effect of the TVC on the outcome at each time point [97]. With extensive development in statistical software and packages in the last decades, it is now possible to test for and fit alternative error structures for the residuals and time-specific variances in MLM models (i.e SAS Proc Mixed, MLwiN, LME, nlme and lmer packages in R). The same holds for the inclusion of TVCs whose effect is not constant across time, which can be dealt with by including residual error structures and TVC by time interactions in the MLM approach [97, 99, 132].

2.3 Person-based approaches: Extensions of the Linear Growth Curve Model

In this section, linear growth curve models are described with multiple outcome variables (section 2.3.1) and with a structured correlation matrix (section 2.3.2).

2.3.1 Multiple outcome variables

In some applications, the research interest may not be restricted to characterising the pattern of change in a single process, but rather to model the change of several outcome variables simultaneously (e.g. cross-domain analysis of change [29]) . To illustrate, suppose a situation where the interest was whether longitudinal change in BMI in childhood can be used to predict insulin level at adolescence, this would require regressing insulin level on the growth rate of BMI in childhood. In such a situation, where intercept and slope factors of the growth curve models are to be used as predictors of some separate distant outcome, LGCM tends to be preferred because it is straightforward to embed the growth curve model into a larger path model specified via SEM[99]. Most MLM software allow the estimation of covariances between growth parameters in multivariate models, but do not allow expanding the structural part of the model directly. Also, SEM can readily estimate all means and covariances between latent growth parameters[97, 107] so that growth models can easily be extended to estimate the interrelationship amongst change patterns in different outcomes, association of change with covariates, and investigate the indirect or mediating effect of covariates on growth processes (see [151]). For example, Breeze et al. 2015 employed latent growth curve modeling to simultaneously estimate trajectories for multiple metabolic risk factors for diabetes (BMI, fasting plasma glucose, systolic blood pressure, and blood lipids), to predict joint correlated risk factors trajectories over a 16 year period [152]. The analysis revealed that change in glycaemia was positively correlated with systolic blood pressure and negatively correlated with HDL cholesterol. As another example of extended growth curve model, Chien et al. used a bivariate LGCM approach to simultaneously estimate the effects of change in systolic blood pressure and serum LDL cholesterol on the trajectory of cIMT, a marker of atherosclerosis, during a 6 year follow-up in a Taiwanese population [56]. It would not be possible to implement such analyses in one-step using the traditional MLM approach to growth curve analysis. However, alternative estimation methods to ML , especially sampling based methods such as

MCMC, can be used for the joint modeling of several longitudinal outcomes within the Bayesian hierarchical framework[153, 154].

2.3.2 Modeling the covariance structure of Growth Curve Models

One of the advantages of person-based growth curve modeling is that it offers great flexibility in modeling the covariance structures for both the between-individual random effects and within individual errors.

Modeling the covariance structure for the between-person random effects

Many MLM or LGCM software consider an unstructured covariance matrix for the random effects (Equation 1.3). As mentioned above, it is the least restrictive but also the most complex structure since all unique elements of the matrix (variances and covariances between all random effects) are free for estimation. While it may be of interest to fit such models to investigates, for example, if one's person outcome level at baseline (i.e. intercept) is correlated with its change in outcome over time (i.e. linear slope), complex growth curve models with multiple random effects may not converge or be overparametrised [114]. One may also wish to explicitly model the variance-covariance matrix to obtain higher explained variances [110]. Most software offer a number of simpler alternative structures for the random effect variance covariance, such as the diagonal, block diagonal structure, which constrain some elements of the matrix to zero. The difference in the -2 Log Likelihood values between two nested models follows a chi-square distribution with 1 degree of freedom. A number of authors discuss the redistribution of the variance between random effects and different likelihood ratio testing strategies to assess the optimal random effect variance structure [110, 114, 121, 122].

Modeling the covariance structure for the within-person random errors

When analysing longitudinal data under the MLM framework, researchers typically assume the within-individual errors to be independently and identically distributed

(i.i.d)) with mean 0 and homogenous variance σ_e^2 [63, 82] .The simplification of the within-individual covariance structure to the identity structure can potentially bias the estimates of the standard error for the fixed effects [63]. By allowing errors to be correlated or residual spread to vary per stratum of a nominal variable (or to increase or decrease along a continuous explanatory variable), the conditional assumption of the MLM model will be relaxed [63]. Researchers can search for optimal variance covariance structure, which theoretically results in higher statistical power and increases the precision of estimates of fixed effects in growth curve models [76, 98, 155]. Choosing the optimal error structure is an important task in MLM, since it is a key element to estimate proper effect sizes and to account properly for missing values [23, 24].

Many authors report that unless highly cyclical, longitudinal data almost always have a proximally autocorrelated structure, since adjacent waves of measurements correlate more highly than adjacent waves, and the more remote in time, the lower the correlation (i.e. time-decaying dependency) [2, 110]. This is the correlation structure used in many LGCM based analyses [97, 107]. For this reason most LGC and software come with the first-order autoregression AR(1) structure for the within-person error by default. This structure contains two parameters, the error variance and the autocorrelation coefficient (see Pinheiro and bates for a matrix representation of the AR(1) [127]). The AR(1) model is one of the few correlation structures that can be expanded to continuous time measurements (i.e. continuous AR(1) or CAR(1)), which is a popular choice for MLM models where the response is a function of age and not measuring-occasion[127]. A number of alternative temporal error structures, such as Toeplitz, and ARMA (if time points are regularly spaced), or spatial error structures (for data measured irregularly in time) are usually available in most MLM software. One possible way to diagnose autocorrelation of residual error is to plot the difference between consecutive level-1 residuals for the same individual against the time difference between the consecutive measures[121, 128]. Another option is to

compare nested models with and without the autocorrelated residual error to decide if the addition to the model is an improvement (by the means of LRT tests) [114, 127] .

Similarly, since standard model formulation does not account for differential variability across population subgroups, it is possible to extend the MLM growth models to allow, estimate, and test for heterogeneous within and between-subject variance terms [66], In fact, Littell et al. warn that failure to account for heterogeneity can lead to inefficient and possibly misleading inferences for fixed effects [156]. The ratio of the between-subject variance to the total variance, also known as ICC (intra-class correlation coefficient) can be used to parametrise random-effect models with varying variance per groups, or trend in the variances across groups [106],[157]. Most MLM and LGCM software implement a number of variance structures that can be used to incorporate varying between- and within-subject variance, including the fixed, identical, power, exponential and combination variance structures [114, 127]. The choice of the variance is based on the nature of the variance covariate (nominal vs. continuous), the presence of one identifiable structure in the model residuals validation plots (e.g. fixed variance structure if residual spread appears proportional to one covariate), or the a priori biological knowledge of a certain type of heterogeneity. However, in most cases it is best judged through model selections using information criteria tools such as the Aikeke Information Criteria (AIC) or the Bayesian information Criteria (BIC) (as in [128]). As for residual correlation structures, the optimal variance structure can be assessed via likelihood ratio test (LRT) if compared growth models are nested (see [110, 115] for more details).

2.4 Person-based approaches: Non-linear patterns of change over time

Many outcome variables collected or measured in longitudinal studies, especially in epidemiology, are characterised by systematic growth processes. As such, some degree of non-linearity in the functional form of the mean response over age or

secular time may be present, particularly if the period spanned by the study stretches across distinct developmental phases for the subject being assessed. Non-linear change is posited by developmental theories in many documented areas of research in psychology and education [70]. While linear change might be a reasonable assumption for short-term processes, the trajectories of many biological and developmental processes over the life course are complex and likely to be non-linear over time [70, 99, 158, 159], particularly if the time-window is large [83, 160]. Researchers must thus consider growth curve models capable of representing non-linear change patterns with a relatively small number of measurement occasions per participant (often <10 time points) and be specific about where between-participant heterogeneity appears in those patterns. Although strategies have been developed to incorporate this potential non-linearity in GCM models in several ways, most examples in the literature present linear GCMs. Specifying an adequate non-linear form of the average outcome over time can be challenging, and may be responsible for the relative lack of uptake of the approach in observational studies where subjects are followed for a long time span [161].

A typical example of non-linear biological measure often collected in observational cardiometabolic related studies, is BMI, a well-established risk factor for metabolic conditions and cardiovascular outcomes [162, 163]. Many large-scale cardiovascular longitudinal studies collect repeated BMI measures on participants over varying study time frames, often with an interest in using BMI trajectory information in childhood or adulthood, rather than BMI at one point in time to predict (or improve the predictions) of later body-size related CVD outcomes [51, 164-166]. BMI typically exhibits separate developmental phases from infancy throughout childhood, as well as from childhood to adulthood, which manifests through non-linear individual BMI-age curves [15, 163, 167]. The successive periods of stable growth, acceleration or deceleration, can not be adequately characterised by a straight line. Also, a lot of between subject heterogeneity in development over time is expected, with some individuals reaching height or weight milestones sooner or later than others, at

different periods in their lives. Similarly, empirical research has shown that blood lipid and blood pressure age-related changes in healthy individuals are not linear across-childhood [168] or adulthood [169]. To properly model longitudinal change in continuous outcomes such as BMI or blood lipids across the life course, the statistical model must be able to accommodate non-linear pattern for the response (i.e. functional form) and allow for between-subject differences in several key aspects of the change in outcome over time, such as difference in starting BMI level, difference in timing of increase in lipid level during puberty, and differences in rates of change in these different periods.

Identifying the shape of the outcome over time is a critical first step in any person-based GCM model, as an incorrect choice of functional form will lead to bias when expanding the initial growth model with predictors of growth or conducting multiple group analysis [97]. Ideally, before any trajectory modeling exercise, the investigator should consider what is known about the development of the outcome that they intend to model [79, 170]. When possible, the model of change should be informed by theory on the development of the system under investigation[25], so that the model parameters can be useful to interpret the longitudinal change and its determinants. In some cases, knowledge on biological processes such as the effect of puberty onset on growth, or the asymptotic nature of human weight and height, can be incorporated in the growth models to obtain an accurate and interpretable representation of change over time. However, for many outcomes there may not be any established theories regarding the form of the longitudinal change, and the optimal functional form of the trajectory over time has to be determined from the data itself [79, 170].

An early approach to deal with non-linearity of the outcome over time was to impose a transformation on either the repeated measure outcome or on age, to make the relationship between the response and time (or age) approximately linear [160]. However, this approach is not very flexible and results in growth curves that are difficult to interpret. In the following section, flexible alternatives that can be used to

model non-linear patters of change over time within individuals in the MLM, LGCM frameworks or both are presented. It is also shown that these can be extended to investigate how and where these patterns change between individuals.

2.4.1 Higher-order polynomial growth models

When a straight line does not appear to be a reasonable model for within-person change in the outcome over time, many investigators often consider a quadratic, or cubic time (or age) parameter to account for the curvature of the response [18, 19, 67, 97, 107]. Following the general notation introduced above, for a P^{th} degree polynomial, the GCM model can be rewritten:

$$y_{it} = int_i + slope_i.t + slope_{2i}.t^2 + \ ... + slope_{Pi}.t^P + e_{it} \qquad (2.7)$$

In the multilevel framework, t^P is the vector of the time-relevant predictor brought to power P, and $slope_{Pi}$, the corresponding random coefficient (i.e. subject-specific P^{th} order growth rate). In the SEM framework, each additional $slope_{Pi}$ is a latent variable with factors loadings values set to t^P [171]. Similarly to what was shown for the intercept and linear slope in the basic linear growth model, higher-order slope terms have means (μ_{slopeP}) and variances (σ^2_{slopeP}) and a full covariance matrix of dimension $(P+1)*(P+1)$. In epidemiology, like in many other fields, polynomial functions such as fractional, orthogonal and natural polynomials, are a popular choice to model continuous individual responses that change non-linearly over time [130, 165, 172]. The quadratic or cubic growth models are often considered first, and if they do not fit the data, a broader range of curves can be considered using fractional polynomials. Briefly, fractional polynomials consist of fitting a series of models using each of eight power of time or age (-2, -1,-0.5, 0.5, 1, 2, 3, where a power of zero is the log function), followed by models incorporating each combination of pairs of these powers [173, 174]. For more complex models, all combinations of multiple powers can also be compared[165]. The best fitting of these fractional polynomial

models is then selected as the functional form, often by comparing the deviance across each model[175].

In general, polynomial approximations of a non-linear function are attractive because polynomial growth models allow testing of known fully-parametric forms of the change over time, which is usually computationally easy to estimate with standard linear modeling procedures. Polynomials have well known and understood properties, and are not dependent on the underlying metric if raw data needs to be rescaled [99]. They allow a flexible range of shapes for the response, in particular non-monotonic responses where the rate of change signs over the data range (i.e. U-shaped curves) can easily be modeled. Polynomial growth models also have a number of limitations such as poor fitting at the edges of the time-window, poor extrapolary properties outside the range of the data, and poor asymptotic properties due to its parabolic change patterns (i.e. they do not accurately reflect trajectories characterised by ceiling effects). This last limitation makes them unlikely to provide a good representation of many biological and developmental outcomes, asymptotic by nature (i.e. many human anthropomorphic measurements as a function of age will tend to a maxima). Additionally, polynomial models exhibit a particularly poor trade-off between shape and degree to model data with a complicated structure, the degree of the model. Thus, if the non-linear trajectory of the response is complex, the number of model parameters, must be high, which often results in highly unstable models and issues of multicollinearity between the many growth parameters. A number of data transformations and modeling strategies exist to avoid these issues [176,177] .

Another downfall of polynomial growth models is that individual higher order growth parameters (such as $slope_{2i}$ and $slope_{Pi}$), can be difficult to interpret, in particular in the presence of interactions. In polynomials GCM, the rate of change is assumed to be constantly changing and higher order polynomial terms can be interpreted as the change to the rate of change, which has little biological meaning. While larger absolute values of polynomial slopes indicate a more rapidly changing growth rate,

resulting in steeper curvatures in the trajectories [107], the estimated polynomial parameters do not have a direct interpretation in terms of the hypothesised change process, since the growth rate is constantly changing. Thus, interpretation is most often based on inspection of plots of average or typical individual growth curves [18, 129], which allows a greater understanding of the estimated model parameters and how they relate trajectories of different groups of participants.

Some strategies allow reparametrizing polynomial growth models so that the estimated parameters are interpretable and reflect important features of the growth curve[178]. That is, the initial level and times at which the individual reached their highest and lowest value for the response [158]. When it comes to determining the optimal order of the polynomial for the measurement model (i.e. level 1 model), parsimony is necessary. A rule of thumb is that at least three data waves per subject is necessary to fit a quadratic within-person model, four for a cubic model and so on. A number of papers discuss formal ways to assess the significance of higher order growth parameters of polynomial growth curves fitted in LGCM [142] and in MLM[115], as well as modeling strategies to include TVC and TIC covariates in higher-order polynomial growth curve models [75, 135], or in fractional polynomial growth curve models [173, 174] .

When using age-term polynomials to describe the observed relationship between the response variable and age, the resulting growth model does not include any theoretical considerations about the underlying mechanism producing the data. The shape of the response is empirically determined, and the models are only designed to detect specific hypothesised overall trends in the time-series, beginning and ending at specified time points [82].

2.4.2 Latent basis growth models

The latent basis growth model [25], also called shape-factor, unspecified [129], or freed-loading [107], is a special case of LGCM, where no constraint is applied to the shape of the trajectory of the within-person response variable over time [117]. It does not have an equivalent in the MLM framework. Because no specific functional form is used, this semi-parametric approach is very flexible and can adequately capture a range of non-linear change patterns. The model can be written:

$$y_{it} = int_i + slope_i.(\alpha_t) + e_{it} \qquad (2.8)$$

All parameters in 1.4 have the same interpretation as shown for equation 2.2, but in this case, $\alpha_t$are known as basis coefficients. The main difference with the general LGC model resides in α_t, which unlike the factor loadings for the linear slope, are estimated from the data itself to closely represent the change process in the response, rather than fixed to progress in a linear manner. To be able to estimate the shape factor $slope_i$, the convention is to set constraints for basis coefficients for at least two time points (e.g. anchor loadings), so that all coefficients in-between can be estimated freely (i.e. fix the first and last points and estimated $\alpha_2$to α_{t-1}). If anchor loadings are set to $\alpha_1 = 0$ and α_T=1, int_i can be interpreted as the subject's level in response at the first time point, and $slope_i$, the total amount of change that occurred between the first and last time points (i.e. t=1 to t=T), but alternative formulations are possible [70]. In this case, estimated basis coefficients $\alpha_2$to α_{t-1} represent the proportion of total change that has occurred up to that point in time. So, as in the LGCM model, the only parameter of change over time is $slope_i$, but here it is not necessarily constant across time, as it changes proportionally with the basis coefficients. Unlike the polynomial growth curve models, one advantage of the latent basis growth model is that it is very parsimonious, regardless of how complex the non-linear shape of the outcome over time. As $slope_i$ will always be the single parameter to describe between-person heterogeneity in the change process, it will have less degrees of

freedom than adding a quadratic or cubic slope to the model [117] . Because the time variable is scaled to achieve optimal model fit, the latent basis model will always be the best fitting model using SEM fit criteria. Latent basis LGCM are often used in case of balanced measurement occasions across the study sample, but they have recently been developed so they can be re-expressed to accommodate individually varying time scores[70]. However, SEM estimation of the basis coefficients remains computationally difficult if designs have many truly unique measurement occasions. Zhang et al. recently presented a Bayesian estimation of a latent basis trajectory model, which they compare with the traditional ML estimation of this model [123]. To summarize, the latent basis growth model is extremely flexible in terms of response shapes, and parsimonious, but like the polynomial model, its parameters can not be interpreted in terms of what is known about the change of the outcome. Additionally, like any SEM model, the latent basis model can not be used for inference outside the sample's study period [99, 117].

2.4.3 Piecewise growth models

A third option for modeling curvilinear growth trajectories over time is to break up the model into several linear or low-order polynomial segments to approximate a more complex underlying form [22, 67, 179, 180]. The distinct 'pieces' or curve segments attach together at knot points, also called 'transition points', or 'change points', so that each segment represents a different phase in the development of the outcome over time, characterised by specific growth factors (i.e. intercept and slopes). Piecewise growth models are semi-parametric models that can be seen as a compromise between the restrictiveness of polynomials and flexibility of latent basis growth models. Because of many possible options in terms of the number of segments, the algebraic form of each piece, and the degree of smoothness at the change points, piecewise growth models are able to model many non-linear processes [181]. Piecewise growth models can be fit in the LGC and MLM frameworks that like polynomial trajectory models, appear to slowly converge in their capabilities, due to

the development of methodological extensions [99]. The choice of framework and estimation method ultimately depends on how one wishes to treat the knots in the model.

Depending on the area of application, piecewise change models are also known as multiphase, linear spline, broken-stick, change point models or segmented growth models [25, 83, 103, 181, 182]. Piecewise growth models are particularly useful if there is an interest in contrasting change rates between different periods, potentially separated by a theorised known or unknown data-driven transition point [21, 24]. Indeed it assumes approximately linear change over limited periods of time, piecewise regression readily allows the estimation and comparison of growth rates in different periods marked by either an expected transitional change, or an experimental intervention point [24]. The model can also be easily extended to accommodate multiple piecewise segments and multiple nodes for complex longitudinal profiles [25].

The simplest piecewise growth model is the linear-linear broken stick trajectory model, also known as two-segment linear spline model. Although alternate parameterisations are possible, a commonly used formulation can be written:

$$y_{it} = int_i + slope1_i.(t - CP_i) + slope2_i.(t - CP_i)_+ + e_{it} \qquad (2.9)$$

Where y_{it}, is the repeated-measure response vector for participant *i* and e_{it} the residual error at each time point. The subscript "+" is an indicator function with $(t - CP_i)_+ = (t - CP_i)$ for t >CP_i and zero otherwise, so that $slope1_i$ is the common linear slope for individual i, $slope2_i$ is the deviation from the initial $slope1_i$ (i.e. slope in second segment is equal to: $slope1_i + slope2_i$), CP_i is the individual-specific transition point between the two linear phases, and int_i is the overall intercept for individual i (i.e. overall response level across all measurement occasions). As shown for the other models above, each intercept, slopes, and change point parameter have their own means and (co)variances (in the case of the linear-

linear piecewise model with random change points, defined a 4 x 4 full-covariance matrix[99]), which can be estimated to investigate average and (co)variation between identified aspects or phases of the trajectories[70,183].

Piecewise growth models can be used to model outcomes that are characterised by documented multiple-phases of growth or development across different life-stages, as the model naturally maps onto what is known about the development of such outcomes over age[163,28, 83, 161]. They are also particularly useful if there is a shift in the outcome trajectory at some point in the event window, such as an acceleration or a deceleration in the change rate of the outcome from one point in time (or age) [83, 180, 181, 184, 185]. Authors have noted that well-fitting polynomial MLM models estimating a non-linear curve can be very useful to describe the average pattern of growth and to assess the relationship between early exposure and later growth. However, they are not conducive to exploring associations between growth and later outcomes, or for comparing growth across populations since the polynomial terms (and their associations with other variables, especially time-varying) are not easily interpreted [83, 103]. In particular, in applications concerned with determinants and consequences of healthy growth and development across different periods in the life course, linear-spline MLM is a useful approach to derive individual summary measures of growth. Linear-spline MLM models overcome several issues such as collinearity of repeat measures and bias due to missing data[175,83] .Linear splines (linear piecewise regression) are the simplest possible type of splines where two or more linear segments are joined (smoothly or not), but more complex non-linear splines (i.e. piecewise polynomials) are also possible both in the frequentist and Bayesian GCM framework. The use of such complex splines function in MLM growth modeling includes notably cubic splines [161] and extended splines functions[186].

In equation 1.5, we presented the most general, but also most complex, form of the linear-linear piecewise growth curve model, where the change point is subject-specific. In some applications, the change point might be treated as an *a –priori*

known parameter, determined by existing knowledge on the system being studied [163, 187]. Sometimes, the aim of the model is precisely to estimate the time (or age) at which the transition occurs in the outcome trajectory over time. In cardiometabolic research, piecewise growth MLM models with predetermined transition points have been used to model BMI profiles from pre-pubertal age to adulthood [163, 187]. The piecewise growth model is particularly suited to model several typical features of normal BMI development across the life-span, such as the rapid linear growth in middle-childhood until people reach their asymptotic adult sizes [188] , followed by a markedly slower increase rate past that point in time [188] that markedly decelerates after puberty once people reach their adult sizes[163, 189]. Because relevant and meaningful aspects of BMI change are directly incorporated as model parameters in the piecewise growth model, the points of interest in the trajectories can be retrieved in one-step, and the researcher does not need elaborate mathematical techniques to derive interesting aspect of growth post-hoc. To illustrate the retrospective way to retrieve meaningful aspects of change in outcome over time *a posteriori* using a more traditional GCM approach, Wen et al. 2012 used a fractional polynomial approach to model individual BMI growth trajectories from infancy to 18 year. As fractional polynomial coefficients do not have a straightforward interpretations in terms of the BMI age-curves, the two childhood turning points of BMI profiles (infancy peak and adiposity rebound milestones) were determined *a-posteriori* based on a change of signs in the first derivative of estimated individual BMI-levels over a range of possible ages, which represented the velocity at each measurement occasion [165].

Piecewise linear trajectory models have been used to model developmental processes with fixed known change points in the frequentist MLM [23, 67, 106, 190] and SEM [107, 129, 148, 185] frameworks, mostly in fields such as education, psychology and intellectual development [191]. Other applications have focused on estimating the change point location in time, notably for the monitoring of longitudinal biological markers where shifts in the outcome trajectory are considered potential indicators of diseases such as Alzheimer, dementia, and some cancers [154, 192, 193]. In the MLM framework,

estimating the unknown (fixed or population-level) change point is well-established, and usually achieved by profiling the log-likelihood over a search grid of possible change points [190, 192], although Bayesian methods can also be used. Some recent work compares the traditional ML and Bayesian estimation methods for a range or piecewise growth models with estimated fixed change points[182]. In the LGCM context, recent papers have also demonstrated techniques to estimate population-average change points [159, 180]. An interesting approach used to select and derive the number and position of knot point for modeling BMI development across childhood, was to use fractional polynomials to derive a smooth function for the curve and then use the derivative of this curve to decide the position and number of change point for the piecewise function [101, 194]. Other options include starting with a large number of knot points and gradually reducing the number until a 'smooth' curve is achieved [83], to place knot points at the centiles of the distribution of age, or use stepwise regression where there is statistical evidence of a difference between linear slopes either side of the knot point[195]. Subject knowledge of the underlying biology of growth parameters may also help in the choice of change point positions, as does the availability of the data (i.e. frequency and intensity of data collection; studies with few measurement occasions may only be able to place knot points at the mean age of each data collection)[175]. Howe et al. 2013 present a very useful suggested workflow for the application of linear spline MLM growth models [83].

In contrast, segmented growth models with subject-specific (i.e. CP_i in equation 1.5) rather than population-averaged change points, are relatively novel and technically challenging, and are the object of ongoing research in the MLM and LGCM contexts. This extension of the piecewise growth model is crucial as it allows individuals to make the transition from one phase to another at different ages or, depending on the design, after different lengths of receiving the treatment or intervention. Between-person heterogeneity in the timing of the transition between growth-phases is expected in many normal or pathological development processes (e.g. inter-differences in age at puberty, differences in age at which cognitive decline

accelerates) [154, 196]. However, it complicates the model estimation greatly. Between-person variability in the timing of the change point between segments was first considered in the MLM framework, by treating the knots as random coefficients in the model [181]. In the general broken-stick piecewise growth model (i.e. linear-linear piecewise model), the absence of a gradient between the successive pieces results in an undefined derivative at the change point making it notoriously difficult to account for random knots using classic likelihood methods [184,190] . Until recently, piecewise growth models with random knots have been investigated mostly in a Bayesian perspective [197], notably in AIDS research [198, 199], cognitive decline and dementia[154, 200, 201]. In the Bayesian context, Congdon 2001 present a thorough presentation of the inference and estimation of piecewise mixed effect model for panel data[125]. Considering a smooth transition, rather than an abrupt one, between the different phases of the trajectory has allowed, in some cases, the traditional ML burden of estimation to be overcome [154]. However, due to its great flexibility and performance at estimating numerous random effects, Bayesian inference still remains popular to estimate a range of 'bent-cable' segmented models with smooth transitions functions for the change point, such as the smooth polynomials, and Bacon-Watts random change point models [153, 202]. Beyond offering numerical advantages for model estimation, smooth random segmented growth models may provide a better representation than the broken-stick model, in cases where an abrupt transition between growth phases is not biologically plausible for the outcome. However, smooth random change point models generally require more time points, and if the data around the real change-point is sparse, the smoothing terms might not be identified or biased [197, 202]. Unless data consists of many available measurement occasions per subject, which is rarely the case in fixed occasions prospective cohort studies, it is advisory to consider the (random) broken-stick growth model and avoid smoothed parameterisations of the model. Recent papers have also developed a method to estimate segmented mixed models with random change point [184] in the ML framework [203].

While a few applications showed how to estimate the change point in the SEM framework, until very recently, it was not possible to estimate subject-specific change points in piecewise LGCMs due to the coefficients entering the model non-linearly. Preacher and Hancock 2015 [204] demonstrate a novel method that allows estimation of knots as random parameters in the segmented LGCM model in a study sample where the complete data was balanced (i.e. relatively low maximum number of possible measuring occasions across the sample). Although promising, the method is complex and involves reparametrizing the model, approximating the function by a first-order Taylor series, and treating the resulting problem as a standard linear model that is estimable via SEM. In consequence, when the sampling occasions are truly individual-specific, or when they are many missing measurement occasions due to a sequential cohort data structure, most authors advise to fit the random change point segmented growth model within the MLM framework using Bayesian inference.

To sum up, the piecewise growth model with or without random change points are very flexible models to represent non-linear change over time and are often preferable to more general non-linear continuous model (such as polynomial) if the number of measuring occasions is small [67]. Although computationally demanding, random change point models are easily fit as Bayesian multilevel (hierarchical) models.

2.3.4 Exponential and other non-linear Growth Curve Models

As opposed to growth curve models illustrated so far (polynomial, latent basis, and piecewise growth models), the 'non-linear' growth curve models that will be discussed in this section refer to growth curve models that follow specified mathematical parametric functions to represent non-linear patterns over time. An example of algebraic non-linear functions is the exponential growth curve, which has been used successfully to model development in language acquisition and cognition using a number of modified formulations, notably a dual increasing-decreasing change over age. [205-207]. Several formulations of empirical sigmoid (s-shaped) curves,

such as Brody, Weibull, Verlhust, logistic, Van Berthalanffy, and Gompertz functions have been used for decades by population ecologists and microbiologists to model processes characterised by an initial period of slow growth, followed by a rapid growth phase and a decelerated growth when the system under study approaches its maxima or carrying capacity [208, 209]. In such sigmoid functions, the instantaneous growth rate is assumed to be proportional to the momentary system's size and the fraction of resources that are still available in the 'habitat'. Apart from biology and ecology, sigmoid curves have been used to describe change in processes such as progress in skill learning [205] in psychology and are routinely used to model dose-response curve in pharmacodynamics and pharmacokinetics [80, 210], and in medicine to describe tumour growth [211]. However, they are still rarely implemented for the analysis of longitudinal data within the growth-modeling framework [170, 212]. The few examples of the use nonlinear functions in growth curve modeling are confined to the fields of psychology and cognition [207]. In epidemiology, an example of such models is one Australian study that used a double logistic function of time to represent the change in HDL cholesterol over time in a sample of peri-menopausal woman measured yearly up to five times as they passed through menopause[213].

In the MLM framework in particular, two authors have contributed to the development of a number of nonlinear mixed models for repeated measure data [80, 127]. In the growth modeling literature, authors further distinguish non-linear growth curves based on how the random coefficients (or latent variable) enter the model. In additive non-linear (or partially non-linear) models[159], subject specific growth parameters are linear in the growth model, while in multiplicative (or fully non-linear) growth, the random effects are non-linear [214]. Consider an additive logistic growth model of the form:

$$y_{it} = int_i + slope_i.\left(\frac{1}{1+e^{-\alpha.(t-\lambda)}}\right) + e_{it} \qquad (2.10)$$

Where, int_i and $slope_i$ are the lower and upper symptote respectively (if the estimated rate of change α is positive), and λ is the time at which α reaches its maximum (i.e. infection point). In this model, the expected outcome values y_{it} for subject *i* are obtained by summing the participant's random coefficients (i.e. int_i and $slope_i$, with means, variances and covariances as in 1.3) each multiplied by a function of time (here $\frac{1}{1+e^{-\alpha.(t-\lambda)}}$) that is invariant across subjects. In contrast, in the multiplicative logistic growth curve models, the rate of change varies between individuals (i.e α becomes α_i) and the model formulation multiplies two random effects (i.e. $slope_i.(\frac{1}{1+e^{-\alpha_i.(t-\lambda)}})$).

Additive non-linear models can be estimated in most MLM and LGCM software, but because a single person-specific parameter (i.e. $slope_i$) controls two distinct aspects of change (rate and total amount of change), $slope_i$ can be counfounding random parameter. Multiplicative non-linear models, although more flexible, can also constitute a non-trivial complication for model fitting due to the multiplication or division between parameters, and need to be solved numerically since they generally lack analytical solutions. Not all MLM or SEM software can handle the estimation of non-linear random effects, which require a number of approximation methods. Grimm and Ram provide an in-depth discussion on the matter, as well as code examples to fit a number of sigmoid growth curve models of both types of growth models in LGCM and MLM frameworks using `Mplus` and `SAS PROCNLMIXED`, which use a number of fitting algorithms [79]. In `R`, non-linear mixed effect models can be fit in `R` using the `nlme` or `lme4` packages, that use an adaptive Gauss-Hermite or Laplace approximation to the log-likelihood[114, 127]. The use of build-in non-linear mixed models in these `R` packages remains quite restricted, with model parameterisations that are not necessarily appropriate for non-linear growth curve modeling. Notably, some popular build-in functions in these packages do not offer to model individual initial response level as one of the model parameter, which can prove problematic when fitting non-linear growth models where this parameter is of

biological interest. A piece of code showing how one can custom-build self-starting functions for the estimation of popular non-linear Gompertz and Richards Growth curve models in R is shown in Appendices 2.1. Compared to similar functions implemented in `nlme` or `lme4`, the model parametrisation in these custom-build functions allows the estimation of the starting level in response as an additional trajectory parameter. In Appendix 2.2, a second annotated piece of code illustrates how these custom-build functions can be used in practice to estimate both the additive and multiplicative form of a given model using simulated sequential-cohort data.

Bayesian estimation of such non-linear models is also possible [207, 215], and the incorporation of prior information or knowledge into the model has proved particularly beneficial in non-linear growth modeling when sample sizes is small.

To summarize, an advantage of non-linear growth model is that there are many possible functional forms so that various complex patterns of change are able to be modeled (i.e. see the `FlexParamCurve` package in `R` that allows to select the best fitting non-linear form among an array of 36 possible monotonic or non-monotonic non-linear models based on double-Richards curves [216]). Because they aim to model the mechanisms giving rise to the observed data, non-linear growth curve models allow extrapolating beyond the observed conditions. Additionally, non-linear GCM generally use fewer parameters than competing linear models, such as polynomial GCM , giving a more parsimonious description of the data, and their parameters are most often easy to interpret since they inform different aspects of the change process. For example, the key parameters used to describe sigmoid curves include the rate of acceleration, the lower and upper asymptotes, the location of changes and the degree of symmetry in the acceleration and deceleration [79]. One of the disadvantages of non-linear GCM is that the fitting process is iterative in nature and usually more complex than for linear GCM models [80]. Convergence issues may arise, particularly with complex-shaped models and multiplicative random effects, and one needs to have an

a-priori idea of values of each growth parameter to allow the model to be estimated (i.e. starting values of parameters). Non-linear GCM are also fundamentally mechanistic, in that they allow for testing specific theory-based hypotheses about change, that is the shape of the response over time is not obtained from the data itself as in other methods, but rather imposed onto the data[217]. While in psychology and development the expected functional form of many longitudinal processes have been well characterised and studied (e.g. the 8 parameters Berkey-Reed 1st order function of age [218] or the 5 parameters Preece-Baines models[219] to represent post-natal human growth in weight and height). In many situations, epidemiologists may not have any prior knowledge or idea on the nature of the change process they intend to model, so have no reason to choose one particular mathematical non-linear function over another[28]. It can prove useful, however, to consider familiar non-linear functions for growth curve modeling when exploring the shape of the response over time. A limitation for using non-linear GCMs in long-term observational studies might be the number of measurement occasions and the number of available repeated measures per participant. Non-linear models need, in general, more data than linear models, and as there can be many random coefficients, it can become difficult to distinguish between different change components when the measurement occasions are sparse [79, 80]. Unstable estimates due to overfitting are a common issue when trying to fit such complex models to sparse data [80].

2.5 Group-based approaches: Growth Mixture and Latent Class Growth modeling

This section considers a second and increasingly popular framework for trajectory modeling that allows the identification of multiple unobserved sub-populations, the description of longitudinal change within each unobserved sub-population, and the examination of differences in change among unobserved sub-populations. In section 2.4.1 group-based approaches to GCM are introduced, and basic elements of the standard Growth Mixture model briefly reviewed in section 2.4.2.

2.5.1 Introduction to group-based Growth Curve Models

GCM approaches discussed so far, MLM and LGCM, have the same central underlying assumption that all individuals come from a single intrinsically homogenous population with common trajectory parameters. That is, that they follow the same basic pattern of change in outcome over time with random variation about that pattern [5, 99, 130]. These approaches are sometimes referred to as " person-based" Growth Curve modeling approaches, since their main feature is the ability to model individual trajectories for each subject for the outcome over time. As discussed in the previous sections, in classic growth curve modeling the shape of the response profile is forced to be the same for every subject in the population, and determined either by existing theory, or by empirical model building. These methods thus assume average trends and express variation in terms of variance around the average response in the population (i.e. with any between-person heterogeneity in these trajectories being absorbed into random growth parameters)[120,129]. MLM and LGCM allow one to test if the predictors are associated with some aspects of the change in the response (e.g. is lower parental education associated with higher sex-and age-specific BMI z-scores at baseline). If the focus is on comparing how response profiles vary between known groups of subjects determined *a-priori* (e.g. sex or grouping based on a distal dichotomous health outcome), the grouping variables are often also treated as predictors of the different growth parameters. Doing so, part of the subject-to-subject variability in different aspects of the change over time is then explained by belonging to one or the other group (e.g. when compared with a reference group, one group may have steeper increase in the response over time). The group variable thus serves to shift the marginal means of the different parameters describing change over time (i.e. intercept, linear change), allowing the description of group-specific prototypical growth curves [21, 97, 107].

In contrast, group-based trajectory modeling approaches have been developed considerably in the last decades to provide a different set of tools to disentangle population heterogeneity and move beyond a 'one size fits all' approach to describing

developmental and change processes across the lifespan [220, 221]. Group-based modeling approaches include Growth Mixture Model (GMM), Latent Class Growth models (sometimes called Latent Class Analysis (LCA) or Latent Class Growth Mixture models (LCGMM)), and semi-parametric clustering analysis [26, 97, 222]. The main goal of these three methods is to identify trajectories for two or more latent classes representing sub-populations hypothesised to exist within the sample, but that can not be observed by direct measurement [223]. Although substantial differences exist between these methods, they assume heterogeneity in the response profiles in the population [26]. Also, they consider that the study sample is composted of a finite number of unobserved, latent categories characterised by similar developmental responses, and that each subjects belong to only one of these latent classes. Instead of subject variation about a single mean growth curve, the growth mixture model has different classes of individuals varying around different mean growth curves [221, 223]. These methods thus different developmental pathways to be identified from the data itself and allows subjects to be assigned to one of the identified trajectories based on their individual profiles.

In Latent Class methods (i.e. Latent Class Growth Modeling / Latent Class Analysis), while different trajectories are identified for a number of classes, subjects within each class are assumed to have identical intercept and slopes (i.e. no within-class variability around the estimated class-specific trajectories)[224, 225]. In contrast, within-class variations are allowed in the estimation of class-membership in growth mixture modeling (GMM), allowing for more heterogeneity within-class[226, 227]. In that sense, Latent Class models can be considered a special case of GMM where all within-class variations are constrained to zero [28]. Due to this absence of random effect in the underlying trajectory parameters, Latent Class models are sometimes considered semi-parametric models, and the approach has been recommended as a way to generate starting values for more complex Growth Mixture models [228, 229] .

2.5.2 Formulation of the basic Growth Mixture Model

The most simple GMM as defined by Muthen [230] can be written as follows: If Y_i = ($y_{1i}, y_{2i}, \ldots, y_{Ti}$) denotes the longitudinal sequence of observations for participant *i* over *T* occasions, let $P(Y_i)$ be the unconditional probability of the sequence Y_i; $P^k(Y_i)$, be the probability of Y_i given membership in class *k;* and π_k, be the probability of a randomly chosen participant of the population belonging to class *k*. The unconditional probability of the observed data Y_i is*:*

$$P(Y_i) = \sum_{k=1}^{K} \pi_k \, P^k(Y_i) \qquad (2.11)$$

where the group membership probabilities π_k are constrained by $\sum_{k=1}^{K} \pi_k = 1$ .This is the sum across all *K* classes of the probability of Y_i given participant *i*'s membership in class *k* weighted by the probability of membership in class *k.* Categorical latent variable c_i represents the unobserved sub-population membership for subject *i*, with $c_i = 1,2, \ldots, K$ and where *c* is the latent class (or trajectory class) variable. The growth parameters and the class membership c_i can also be influenced by covariates. Here, time-invariant covariate *x* is considered, and a multinomial logistic regression model is used to predict the latent class variable *c* by the covariate *x* for each of the *K* classes.

$$P(c_i = k|x_i) = \frac{e^{\delta_{0k} + \delta_{1k} x_i}}{\sum_{s=1}^{K} e^{\delta_{0s} + \delta_{1s} x_i}} \qquad (2.12)$$

The GMM considers separate growth models for each of the *K* latent classes with difference in all possible trajectory parameters (i.e. fixed effects of intercept and slope(s)). It can also be extended to predict a categorical distal outcome where the latent trajectory class is used with the covariate *x* to predict the outcome by the means of logistic regression.

A particularly attractive feature of this group of methods is that the influence of covariates or predictors can be considered in a number of ways. For example, as part of each participant's profile description, as predictors of class membership, or as predictors of growth parameters for each class.

Firstly, identifying characteristics that make trajectory group members distinct form other trajectory groups can be done using posterior probabilities of class membership. These probabilities represent a measure of a participant's likelihood of belonging to each of the *k* classes of trajectories. Posterior probabilities of class membership are computed post-model estimation, using the coefficient estimated when fitting the GMM [220]. For a given model, each individual obtains a posterior probability estimate for each latent class computed as a function of the model parameter estimates and each participant's observed values using Bayes' theorem[225]. For each individual, the k^{th} class with the highest posterior probability determines the individual's likely trajectory class membership. This measure can also be used to access the discrimination of a given model to see if the model clearly differentiates in which class participants belong. Ideally, average posterior probability of at least 0.7 for all groups indicates that the model provides good class discrimination [225] . These measures of class membership can be used to investigate the subject-level characteristics, which may be associated with belonging to one or the other trajectory group, allowing to "profile" people within each group. This can be particularly useful in trying to determine determinants that will identify people most at risk for following deleterious trajectories of risk factors or health markers over time [226, 231, 232].

Secondly, the GMM and latent class models can include both TICs and TVCs covariates, where they act as adjustments to the intercept or interaction with the slope parameters [225,233].

Thirdly covariates can be introduced as predictors of trajectory group membership by the mean of a multinomial logistic regression model specifying the functional

relationship between the probabilities of class membership π_k for the k^{th} group of K groups, and a set of M predictors x_{mi}, estimated simultaneously with the trajectory parameters [118]. The probability of belonging to class k can be defined as:

$$P(c_i = k|x_{mi}) = \frac{e^{\delta_{0k}+\delta_{1k}x_{1i}+\cdots+\delta_{mk}x_{mi}+\cdots+\delta_{Mk}x_{Mi}}}{\sum_{s=1}^{K} e^{\delta_{0s}+\delta_{1s}x_{1i}+\cdots+\delta_{ms}x_{mi}+\cdots+\delta_{Ms}x_{Mi}}} \quad (2.13)$$

Where the logistic regression parameters δ_{mK} are set to zero for the last class K (i.e. the reference class).

For *k=1:*

$$log\left[\frac{\pi_1\,(x_{mi})}{\pi_2\,(x_{mi})}\right] = e^{\delta_{01}+\delta_{11}x_{1i}+\cdots+\delta_{m1}x_{mi}+\cdots+\delta_{M1}x_{Mi}} \quad (2.14)$$

2.5.3 Aspects of Growth Mixture Modeling and recommendations

The identification of predictors of group-membership is a particularly interesting feature of this class of models for cardiometabolic research, since it can potentially inform prevention intervention. For example, it allows investigating whether parental obesity is associated with the probability of belonging to BMI trajectory groups with differences in overweight/obesity onset mechanisms over time. There has been discussion in the literature as to whether TIC covariates should be included in the trajectory part of the model or the logistic prediction of group-membership part. In general, when trying to include these predictors in both, the variable becomes a non-significant contributor of group membership, such that covariates in the predictor part of the model should be restricted to those measured at baseline only [233 225].

A frequent critique of group-based approaches to growth curve modeling is that different model specifications of the within-class random structure can lead to very different models derived in terms of the "best" number of growth mixtures, their size, and their composition. In general, more latent classes will be required to represent the

population heterogeneity when fitting Latent Class compared to Growth Mixture models [226, 231]. When possible, the underlying random structure in these types of models should be informed by, and understanding of, the underlying cause of variation. It is thus necessary to note that the results of such methods must be interpreted with caution, and in full knowledge of how the model was specified, especially since the nomenclature used to describe the modeling approach can be confusing or misleading.

There are a number of statistical indices that were developed to select the 'best' Growth Mixture model [227 221, 223, 234] . The methods used to determine the number of classes which best represent the data for frequentist models include the likelihood ratio test [235], the BIC that captures competing models parsimony by adjusting the likelihood by the number of parameters and sample size, the Lo-Mendell-Rubin likelihood ratio test [236], and the bootstrap Likelihood Ratio Test (BLRT) [237]. Although simulations studies indicated that the BLRT and BIC are the most reliable methods to assess GMM models [227], their usefulness is sometimes limited in practice, in particular in situations where these indices indicate that increasing the number of classes past 6 or 7 keeps improving model fit [28]. In general, many authors recommend that researchers need to draw a balance between model complexity and interpretability, since the selection of the optimal model is not always straightforward.

In summary, although a number of theoretical and technical issues may arise with group-based approaches to trajectory modeling, compared with methods that assume that all subjects follow the same functional form (i.e. person-based MLM and LGCM growth curve models), partitioning the variability in this way may allow for a better understanding of change in response over time and provide another means of detecting critical periods, which can complement the classic individual-based approach to growth curve modeling. Indeed, person-based trajectory modeling such as Latent Class Analysis, or Growth Mixture modeling may reveal the existence of

potential sub-categories within a population that one may not have been able to detect when the grouping of the curves was determined *a priori.* In cardiometabolic research, group-based approaches to trajectory analyses have allowed epidemiologists to study the potential heterogeneity in the development of BMI to identify different mechanisms of obesity incidence and their determinants in a number of populations. In particular, several studies have prospectively explored BMI trajectories in the periods of early childhood crossing over adolescence, using raw BMI, or BMI z-scores [238]. This approach provides an opportunity to identify distinct underlying mechanisms of obesity resolution in the population (i.e. early onset resolution vs. late onset resolution), which were not feasible using traditional MLM or LGCM approaches. Being able to identify distinct age-related trajectories among incident obese or resolving obese subjects is crucial, especially if predictors of latent class membership can be determined. This could help identify children at higher probability of becoming obese at earlier or later points in life, which may further help refine and target obesity interventions suggested by the individual-based GCMs.

In addition, since it provides an empirical means of identifying clusters of individuals following both typical and atypical courses of risk factor development, the analysis of developmental outcome trajectories using a group-based method may be used as a confirmatory approach to judge the appropriateness of the subject-based GCM strategy (i.e. MLM/LGCM models). Even in situations when the primary interest might lie in contrasting outcome trajectories between groups based on predetermined groups of participants, the group-based approach can be used to assess the concordance between estimated latent trajectories and those modeled in the groups of interest, as well as get a sense of whether the chosen functional form in the GCM can be used to describe all trajectory groups [226, 231].

2.6 Summary

This chapter has provided the methodological foundation and background of this thesis. It reviewed specific aspects that pertain to Growth Curve modeling of continuous longitudinal outcome data arising from long-running cohort studies in epidemiology. More specifically, the difference between person-based and group-based growth curve modeling approaches, and the different techniques that allow estimating these types of models was reviewed. A number of modeling strategies that accommodate for potential non-linear responses over time within-person (or age) was presented within each framework, as well as critical aspects of study design and specific research questions that should be considered to guide the choice of modeling approaches in different practical situations. In particular, when the continuous response data arises from accelerated cohort designs with less than 10 repeated-measures per participants, Table 2.1 and 2.2 provide a visual summary of the type of analyses, advantages and drawbacks of each Growth Curve modeling approach reviewed in this chapter.

In addition, by (1) Identifying, reviewing and compiling the key technical difficulties and issues that remain when implementing the different existing growth curve modeling strategies to sparse continuous longitudinal data, such as those arising from long-term observational studies, and by (2) showing the lack of appropriate methodology to investigate group-divergence in non-linear trajectories of continuous response overtime, this chapter introduces the specific aims that the thesis will be addressing (aims 1 and 2), i.e. the tailoring, extension and modification of existing growth curve modeling theory for the analysis of accelerated longitudinal designs or multi-cohort studies, and the development of a novel statistical tool approach to identify the point in the life course at which participants from different groups of interest start to diverge in their non-linear trajectories of continuous response overtime."

Table 2.1. Summary table of applications, statistical assumptions advantages and drawbacks of person-based Growth Curve Modeling approaches reviewed in Chapter 2

	Applications	Features/Assumptions	Framework	Form of change	Advantages	Disadvantages
Person-based Growth Curve Modeling	- Estimate inter-individual variability in intra-individual patterns of change. - Find determinants/ predictors of between-person difference in response profiles. - Test the association between individual-or group-specific change in outcome over time and realisation of a binary health outcome in later life (i.e. one-step analysis (LGM) or two-step analysis (HLM/MLM)).	- Estimates individual and population-average trajectories. - Possible to model simultaneously trajectories in multiple *a-priori* groups (e.g. sex, family history). - 'Initial status' and 'change' modeled as growth parameters (HLM) or latent variables (LGM) (i.e. intercept/slopes), and can be related to predictors. -Specific form/shape of time- or age-related change is determined empirically from data (population-average response). - All persons in the population assumed to follow the same pattern over time (i.e. heterogeneity around average curve captured by random effects). - All person-based models can be estimated in the frequentist or in the Bayesian framework. - Handle ignorable missing data (MAR/MCAR).	**Latent Growth Modeling, (LGM)** (special case of Structural Equation Modeling) - Requires outcome measured approximately at the same age for all cohort members. **OR:** **Hierarchical /Multilevel Linear Modeling (HLM/MLM)** - Easily handles unbalanced time-spacing data structure and dependency among observations. - Best	**Polynomial Growth Curve**	- Very flexible to model a range of complex non-monotonic within-person profiles over time. - Useful to explore the non-linear shape of the response among groups of interests.	- Polynomial coefficients difficult to interpret biologically. - Poor fit at the extreme, numerically unstable if the pattern of change is complex. - Does not allow a direct estimate of age at divergence between groups.
				Non-linear Growth Curve	- Many forms to choose from, including complex semi-/non-parametric functions (such as splines). - Common non-linear functions can adequately represent asymptotic change within-person over time -Easier to interpret than polynomial GCMs.	- Larger number of repeated measures per person (>10) needed to fit most curves (many estimated parameters per person). - Convergence issues common. - Choice of form must be guided by biological knowledge. Can only model monotonic change over time. - Does not allow direct estimate of age at divergence between groups.

		- Time-independent variable predict the growth parameters. Time-varying predictors predict observation level-error at each time point.	framework to estimate Growth models with cohort-sequential designs.	**Piecewise Growth Curve** (aka: linear spline, segmented, broken-stick growth curve)	- Allows modeling multiphase processes using meaningful growth parameters. Possible to compare growth rates at different periods. - Easy to interpret, often map onto known aspects of outcome development. - Fit better than polynomial curves with few data points.	- Computer intensive. - Biological theory needed to guide demarcation of successive phases. - Approximate estimation methods needed if change points between successive growth phases are to be modeled as random parameters (i.e. subject-specific change points).

Table 2.2 Summary table of applications, statistical assumptions advantages and drawbacks of group-based Growth Curve Modeling approaches reviewed in Chapter 2

	Applications	Features/Assumptions	Advantages	Disadvantages	Type of models
Group-based growth curve modeling	- Identify distinctive subgroups (latent classes) *a posteriori* in the population that follow different change patterns over time. - Estimate class sizes and participant characteristics within each trajectory class. - Identify predictors of latent class membership (i.e. multinomial logistic regression). - Test whether class membership predicts a distal health outcome (i.e. logistic regression).	- Estimates individual trajectories over time. - Mixture model: the study sample is a mixture of latent (unobserved) subpopulations. - Specific functional forms for different groups within the population are estimated from the data itself. Type of (non-linear) change needs to be specified in advance (i.e. linear, polynomial, piecewise forms). - All group-based models can theoretically be fit in the frequentist and Bayesian framework (although Bayesian framework is less developed at present). - The probability of belonging to each latent class is generated for each subject. - It can handle ignorable missing data (MAR/MCAR). Suitable for cohort-sequential designs	- Allows the identification of different developmental pathways in the outcome in an underlying population that would be considered as error variance in person-based methods. - Straightforward to test association between patterns of change over time (latent classes) and distal outcomes in one stage.	- Computer intensive. - Convergence is often an issue. - Model building is not straightforward: many increasingly complex models need to be run and compared. - Some degree of arbitrariness in model specification and difficulty to select the optimal model (despite several statistical indices that can be used to decide of the best model) - Need to draw a balance between model complexity and interpretability. - Different model parameterisations can result in very different interpretations (i.e. # of classes and composition). - Crucial to specify the random structure using biological understanding of causes of variations.	**(Latent) Growth Mixture model (LGMM)** - Within-class variance estimated for each latent class. - Possible to compare different within-class variance structures. **Latent Class Analysis (LCA)** - Special case of LGMM with within-class variance is constrained to 0. **-** More latent classes than in LGMM are usually required to capture population heterogeneity since no random effects estimated for each class.

3. THE COMBINED EFFECT OF COMMON GENETIC RISK VARIANTS ON CIRCULATING LIPOPROTEINS IS EVIDENT IN CHILDHOOD

3.1 Preface

In this chapter, the aim was to demonstrate the applicability of person-based multilevel growth curve models specified as higher-order polynomial function of age to analyse non-linear continuous responses across the life course with frequentist methodologies. This approach is useful in situations where there is no existing biological theory to guide the specification of the functional form of the response over time *a priori*. Individual-trajectories of circulating blood lipid levels from age 6 to 49 years old in the YFS cohort are modeled and the combined effect of identified risk alleles on the development of blood lipid trajectories from childhood- to adulthood are investigated. Specifically, this chapter illustrates how to examine the combined time-averaged and the time-dependent effect of GWAS-derived genetic risk scores on the atheroprotective HDL-C, and on LDL-C, and triglycerides (TG). Additionally, how to test for effect modification within the MLM framework is demonstrated by investigating whether the multigenic predisposition to adult dyslipidemia might be modified by the magnitude of BMI change from childhood to adulthood, used as proxy indicator of trajectory of lifestyle-related factors.

In summary, in line with general aim 1 and 3 of this thesis, this chapter illustrates how careful implementation of MLM growth curve modeling can help gain insight into the complex influence of genetic factors on developmental trajectories of lipid protein.

3.2 Introduction

Cardiovascular disease (CVD) is the leading cause of death and a major health burden worldwide [239]. Although modified by diet, obesity, lifestyle and other environmental factors, circulating lipoproteins represent a crucial partly heritable risk factor for atherosclerosis and CVD[57, 240]. Notably, elevated levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C), have shown association with preclinical atherosclerosis in children and

adolescents [42], significantly contributing to adult atherosclerosis. LDL-C, in particular, plays a major role in the initiation and progression of atherosclerotic lesions [241, 242]. The relevance of high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) to cardiovascular risk has been extensively debated in the last two decades. Although recent findings have questioned the causal relationship between genetically-defined HDL-C levels and atherosclerosis [243, 244], numerous prospective and case-control epidemiological studies have reported an inverse association between HDL-C levels and the risk of CVD[17, 245, 246]. Low HDL-C is therefore considered an independent risk factor for an increased risk of coronary artery disease, although pathways to its potential antiatherogenicity, are still not well understood [247]. High TG levels are markers for several types of atherogenic lipoproteins involved in atherosclerosis. In contrast to LDL-C, the epidemiologic evidence-base for elevated TG as a CVD risk factor is generally less clear [248-250]. However, recent evidence suggests that genetically-defined plasma TG levels are associated with coronary artery disease risk, even after correcting for confounding effects due to LDL-C or HDL-C levels[251]. In light of these associations, managing dyslipidemia remains a continuing trend both in primary and secondary prevention and risk reduction of CVD worldwide[252].

While the greatest deviations from normal levels of lipoproteins are principally monogenic, the majority of adverse circulating lipid profiles arise as polygenic disorders with a substantial environmental component (e.g. diet, smoking, obesity)[253]. Although dyslipidemia is common, the complex interplay between various genetic and environmental risks that lead to onset and progression of the condition are still poorly understood. In the past 10 years, multiple genetic linkage analyses, candidate gene analyses, and large-scale genome-wide association studies (GWAS) have pinpointed a number of common genetic variants of candidate genes associated with inter-individual variation in plasma lipid levels [240, 254-259], indicating a genetic predisposition to dyslipidemia. Most lipid-associated SNPs (single nucleotide polymorphisms) are characterised by relatively small effect sizes, however some of the reported loci contain genes of clear biological and clinical importance, implicated in established mechanisms of lipoprotein metabolism [260]. Because most individual risk variants only explain a small fraction of those traits' heritability, the development of multilocus genetic risk scores that combine or accumulate the influence of validated susceptibility markers have proliferated in the hope of improving genetic CVD and other disease risk prediction [261-263].

Unfortunately, it is currently largely unknown whether reported lipid-associated risk alleles have any clinical relevance for a genetic predisposition to elevated adult or childhood lipid levels. With most GWAS leveraging cross-sectional samples from middle-aged adult populations, the relative contribution of these genetic factors to the early stages and development of dyslipidemia between childhood and adulthood remains poorly understood[264] [265] [266]. Moreover, whether identified risk alleles or GWAS-derived genetic risk scores predict lipid trait levels at all ages or whether they are associated with the intra-individual progression of serum lipids over time is unknown.

The combined time-averaged and time-dependent effect of validated genetic risk alleles on lipoprotein trajectories across the life course was examined in the YFS Study. Tikkanen et al. have reported population specific cross-sectional associations of 95 GWAS-derived individual SNPs with lipid levels in the Finnish cohort [267]. However, no study to date has investigated the association between lipid genetic risk scores and the development of lipid-trajectories across the life course at the individual level. The primary aim was to quantify the contribution of a multigenic lipoprotein score to elevated levels of the LDL-C (14 risk SNPs) and triglycerides (24 risk SNPs) as well as depressed levels of the atheroprotective HDL-C (38 risk SNPs) from childhood to adulthood. It was also investigated whether a multigenic predisposition to adult dyslipidemia might be modified by a lifestyle trajectory indicator such as the magnitude of BMI change from childhood to adulthood.

3.3 Methods

3.3.1 Participants

The YFS tudy is an ongoing population-based prospective cohort that started in Finland in 1980. It was designed as a collaborative effort between the five medical schools in Finland to investigate cardiovascular risk factors and their determinants from childhood to adulthood [62]. A varying number of participants from the original cohort (consisting of 3596 children aged 3 to 18 years in 1980) were measured through middle adulthood (maximum age 49 years) in 2011 for numerous traits related to CVD development, and have as many as seven follow-up measurements (Tables A and B in Appendix 3.1, Figure B in Appendix 3.2). Among the traits measured at multiple times, serum lipoproteins (plasma concentrations of LDL-C, HDL-C and TG) were collected at baseline and all seven follow-ups. For this study, analyses of the

association between polygenic risk scores and circulating lipoprotein trajectories from 1980 to 2011 were pursued. These analyses included up to 2442 participants for whom genetic information was available for each of 76 risk SNPs identified in the literature. Participants reporting use of lipid lowering medication in 2001, 2007 and 2011 were excluded from the analyses (n= 7, 46 and 77 participants excluded respectively for the analysis of LDL-C, HDL-C and TG life course trajectories). Participants or their parents provided written informed consent, and the study was approved by local ethics committees (The Ethics Committee of the Hospital District of Southwest Finland) in agreement with the Declaration of Helsinki.

3.3.2 Measures

Blood lipid and lipoprotein measurements

All serum lipid determinations were performed in duplicate on fasting samples using standard methods in the same laboratory. HDL-C was determined enzymatically after precipitation of very low-density lipoprotein and LDL-C with dextran sulfate 500 000 (Olympus System Reagent, Olympus Diagnostica, Hamburg, Germany) in a clinical chemistry analyzer (AU400, Olympus Optical, Mishima, Japan) [268]. The concentration of LDL-C was determined indirectly by the Friedewald formula, so those participants with triglycerides >4.0 mmol/L (n=32) were not included in the LDL-C evaluation [269]. Specific details on the lipid determination methods used in earlier [270] and later [61] follow-up studies have been published previously. To adjust for changes in kits and determination methods across study years, lipoprotein levels from early follow-ups were corrected to those measured in the most recent follow-up using correction factor equations, which were determined with linear regression analysis utilizing standardised principal component adjustment [61, 271]. For each lipid, the specific calibrating equations and the dates at which analyzers and reagent suppliers changed along study years are presented in the Appendix 1 of [270] and in [271]. HDL-C profiles were the focus of this study because measures of other HDL-C fractions were not available at all time points in the YF study sample.

Adverse levels of lipoproteins (i.e. abnormally high LDL-C/triglycerides, and low HDL-C), which identify participants at increased risk of developing atherosclerotic CVD (normal risk vs. high risk), were defined using the National Cholesterol Education Program (NCEP) adolescent and childhood cut points (for participants under 19 years) [272] and NCEP adult-

treatment panel guidelines (for participants 19 years old and older) [273]. The NCEP has not defined desirable and undesirable TG levels for children and adolescents, so high risk TG levels are classified using cutoffs suggested in AAP and AHA pediatric guidelines [274] . The cut points used to define 'normal' and 'high risk" lipid levels are shown in Appendix 3.6.

Genetic measures and genetic risk scores

In the 2001 follow-up, a, subset of original participants (1,123 males, 1,319 females) were successfully genotyped using a custom Illumina BeadChip containing 670,000 SNPs, for a final list of 546,677 SNPs that passed quality control and allele frequency filters. The exact custom content of the probes, as well as initial clustering, filtering, and data exclusion are described by Smith et al. [260]. Genotype imputation was performed using MACH[275] with the HapMap haplotypes as a reference panel (phase II, release 22 CEU, NCBI build 36, dbSNP 126). In the present analyses, we used 38 HDL-C, 24 TG and 14 LDL-C associated SNPs identified by genome wide meta-analysis conducted by the Global Lipids Genetics Consortium (GLGC) on 46 lipid GWAS carried out in over 100,000 European individuals of Caucasian descent [258]. Three composite genetic risk scores (wGRSs) (LDL-C, HDL-C and TG wGRS) were constructed as the arithmetic sums of these 38 HDL-C lowering, 24 TG-raising and 14 LDL-C raising alleles respectively, weighted by their effect sizes (in $mg.dl^{-1}$) as established from a published large-scale meta-analysis [258, 263] (Appendix 3.5). To avoid redundancy and overlap of genetic information, in each lipid wGRS, only the SNPs that showed the strongest independent associations among the 3 lipid traits in the meta-analysis were included[258]. The variant rs9411489 was not included in the LDL-C wGRS because it was missing on the chip and not available in the HapMap 2 reference panel. For comparability of metrics and to estimate the wGRSs ability to discriminate between extreme lipoprotein phenotypes, participants were categorised into 'high' and 'low' genetic risk groups categories defined as the cohort-specific lower (25^{th} percentile) and upper (75^{th} percentile) quartile of each composite risk score variables (HDL-C wGRS, TG wGRS and LDL-C wGRS). In each case, the remaining 50% of participants, lying in the interquartile range, were classified as 'medium' genetic risk (Table 3.1). Histograms of each lipid's wGRS are presented in Figure A in Appendix 3.2. This approach is commonly preferred to case-control dichotomy when investigating the association between genetic factors and disorders implicating quantitative traits continuously distributed over the population (such as

dyslipidemia), because it increases the statistical power of testing the variants for association [276].

3.3.3 Statistical analyses

Association between longitudinal lipoprotein profiles and composite genetic risk scores
The principal outcome was the association between the categorical polygenic risk score status (High vs. Low wGRSs) and longitudinal trends in HDL-C, LDL-C and TG levels from 1980 through 2011. To determine whether sex and genetic risk group membership modifies average lipoprotein level or the growth parameters of the participants' lipoprotein trajectories over time, individual growth curve analysis (IGC) was used. IGC is an advanced multilevel mixed effect regression technique that allows the simultaneous modeling of inter-individual differences in intra-individual systematic changes over time (i.e. repeated individual measurements) [18, 19, 23, 97]. An IGC model comprises 3 main components: (A) the functional form of the response variable, which partitions and quantifies the variance across people and time, (B) the fixed effects (i.e. group-level predictors of change), and (C) the stochastic part of the model, which includes the random effects (i.e. individual effects on growth parameters), and the residual error covariance structure. While there is, in the literature, a few variants in the specification and the procedure of IGC model building (sometimes also referred to as 'growth curve analysis' (GCA)[18]), the modeling strategy suggested by Singer and Willett [23] was followed, with a few adaptations (see Appendix 3.8. for a step by step protocol of the modeling approach, and explicit parametrisation of the IGC submodels).

Prior IGC analysis, individual empirical growth plots and generalised additive mixed models (GAMMs) were used to explore the functional form (shape) lipid profiles across the life course in the YFS cohort [277] and inform the modeling procedure. For each lipid, the IGC analyses then consisted of testing several submodels as follow: (1) an unconditional mean (UM) model (i.e. null model), examining any difference in average lipid levels between individuals, (2) a linear unconditional growth (UG) model (with no group-level predictors), used as a reference to explore the functional shape of the lipid growth over time (3) two or more higher-order polynomial UG models to test if the lipid rate of change was accelerated or decelerated as subjects aged (i.e. curvilinear age-related change), (4) models for slope(s) variability, to test for random trajectory parameters between participants, (5) a set of models

to assess the within-subject error structure of the best UG model to test if incorporating (a) an autoregressive structure with serial correlation, and (b) heterogeneity of the residual error will improve model fit, and (6) a conditional growth (CG) model, where wGRSs, sex and their cross-product are introduced as subject-level predictors of each growth parameter variability (i.e. intercept, linear -, quadratic- and cubic (and quartic) growth). CG models allows assessing average wGRSs effect effect at baseline and whether or not there was an age-dependent effect of wGRS score on each lipid's trajectory parameters (i.e wGRS*age, wGRS*$(age)^2$,…) . CG models also examine whether individual variability in lipid intercept and slopes estimates can be accounted for by the interaction of wGRSs and sex. Throughout the IGC analyses, when comparing increasingly complex submodels, the improvement in model fit is assessed by likelihood ratio test (LR-test) or using Akaike's and Bayesian Information Criterion (AIC and BIC). The significance of each estimated model growth parameter in the final CG model is assessed with t-statistics (i.e. defined as the ratio of parameter estimate and SE) (Appendix 3.8). A flowchart of the IGC modeling approach is outlined in Appendix 3.4.

Prior to introducing sex and wGRSs as time-independent predictors of individual variability in lipid trajectories, consideration was given to minimise for confounding by (1) birth cohort and (2) period effect. We tested whether "year of birth", calendar "year" at examination or both modified the age-related trajectory of lipids across the life course. This was performed by adding the variables (1) birth year ("yob", categorical variable with 6 levels) and (2) year at follow-up ("year", centered around baseline (1980)), and their interactions with trajectory parameters to each lipid's sex-specific UG model (Appendix 3.8). Birth cohort does not appear to significantly modify the lipid profiles across the life course in this study sample (i.e. later birth cohorts do not show significantly different lipid trajectories compared to earlier birth cohorts). However, as significant linear yearly secular trends were found for each lipid (see Results section 3.4.1), "year" was adjusted for in all subsequent steps of the IGC analyses.

HDL-C and LDL-C distributions were reasonably close to normal (Appendix 3.9). Because TG levels showed very long tails skewed to the right, we applied the Box-Cox procedure to determine the optimal transformation to remediate deviations from the assumptions of the linear regression model [278]. As the best transformation (λ=-0.2) was close to the logarithmic

case, natural logarithm was used to transform triglycerides levels prior all analyses. Ages of participants at each measurement were treated as continuous covariates, and centered around youngest age at baseline (3 years old) to avoid collinearity problems with higher-order polynomial age terms and their interactions in the multilevel mixed models [85]. As an index of fit of the different linear mixed effect models (i.e. final CG models), and to estimate how much the genetic predictors contribute to the variation of the lipoprotein profiles outcome, the novel conditional R^2 and marginal R^2 for linear mixed models developed by Nakagawa & Schielzeth, 2013 [279] and adapted by Johnson, 2014 [280] was used to accommodate for random slopes. For a given mixed effect model, the marginal R^2 describes the proportion of variance explained by the fixed effects alone, while the conditional R^2, describes the proportion of variance explained by both the fixed and subject- level random factors. In the case of IGC models, which are typically hierarchical mixed models, these two novel coefficients of determination are superior to the pseudo-R^2 often reported for linear mixed model (i.e. squared correlation between the fitted and observed values) which ignores the variance components at multiple levels of the random factors by choosing to calculate R^2 relative to only the residual variance. All analyses were performed in `R 3.0.1 9` [281] using the `nlme 3.1.102` [114] and `mgcv` [282] packages.

To complement the categorical analyses and make inferences at the population levels, a similar age- and sex- adjusted mixed modeling growth curve analysis approach was used to examine the association of the continuous wGRSs and the longitudinal trends of HDL-C, LDL-C and triglycerides. As per above, main effects (age-averaged) as well as age-dependent effects of continuous lipoprotein risk scores on lipoprotein trajectory parameters were assessed. Z-scores were calculated for the wGRS prior to these continuous analyses so that for LDL-C, HDL-C, and, TG the estimated effects (i.e. the regression parameters βs) indicate the change in mmol/L lipoprotein level per 1-sd change in wGRSs. For triglycerides, the regression coefficient βs were exponentiated for ease of interpretation, so that exp(βs) correspond to changes in the ratio of the expected triglyceride level per1-sd change in wGRSs.

Secondary analyses

Age- and sex stratified linear regression analysis

To ascertain the age at which the polygenic effect on the lipoproteins is first detectable and examine the strength of association between continuous genetic risk scores wGRSs and lipoprotein levels across age groups, sex-specific age-stratified linear regression models adjusted by study year were used. Trends in the associations between age-groups were assessed using LOESS curves. Additionally, a table summarizing sex-specific associations between categorical genetic risk scores and lipid levels at ages 3 years, 15 years, 24 years and 45 or 46 years is presented (Appendix 4.7). The reported mean effect sizes are in mmol/L for the number of risk allele differences between high and low wGRSs for each lipid.

Indication of polygenic gene-lifestyle interaction on adult lipoprotein

Because lifestyle factors relating to weight status (i.e. dietary, exercise, and sedentary habits) are known to strongly correlate with blood lipids, it was determined whether the polygenic effect of risk loci on adult lipoproteins might be modified by an individuals' BMI trajectory from childhood to adulthood. For this, we test for an interaction between continuous wGRS and change in standardised BMI between childhood and adulthood, ΔBMI_i, in sex- and age-adjusted linear regression models of adult lipoproteins (n=2100 for adult LDL-C model, n=2062 for adult triglycerides, and n= 2034 for adult HDL-C model). For each participant *i*, this measure was calculated as:

$$\Delta BMIi = z.score\ BMI_{(adult)i} - average\ (z.score\ BMI_{(Childhood)i}) \qquad (3.1)$$

with z-score $BMI_{(adult)}$ defined as the sex-specific BMI z-scores observed at the latest follow-up attended in adulthood for adults 30 years old or older in 2001, 2007, or 2011 (i.e participants younger than 30 years old in 2001 were excluded) ; and average(z-score $BMI_{(childhood)}$) defined as the participant's average of sex-and age-specific BMI z-scores measured at multiple occurrences in childhood (ages at follow-ups ranging 3 to 18 years). The significance of the interaction term (wGRS *Δ BMI) as a predictor of either adult lipoprotein levels was assessed by likelihood ratio test.

3.4 Results

Participant characteristics for lipoprotein levels and genetic risk scores considered in the longitudinal analyses are shown in Table 3.1. The difference in the number of risk alleles

between subjects in the high wGRS and low wGRS group ranged between 4 and 7 alleles on average for LDL-C and HDL-C, respectively (Appendix 4.7). When stratified by life stage (i.e. childhood (ages 3 to 15 years), young adulthood (ages 18 to 27 years) and middle adulthood (ages 30 to 49 years), average concentrations of each of the three lipoproteins were mostly consistent with pediatric and adult healthy cholesterol and fasting triglycerides levels (NCEP guidelines 2010), and the standard deviations were homogeneous over time. For each lipid, a histogram of the continuous wGRSs distribution also showing the quartile stratification into 'low', 'mid' and 'high' genetic risk is shown in Figure A in Appendix 3.2. Additional descriptive statistics showed that the wGRSs were not strongly linearly correlated with the lipoproteins overall when ignoring the effect of participants age (*Pearson's r* = 0.21 for LDL-C, 0.19 for HDL-C and 0.18 for triglycerides).

3.4.1 Longitudinal lipoprotein profiles

Significant decreasing secular trends were observed for LDL-C and TG between 1980 and 2011 ($\beta\ year_{LDL\text{-}C}$=-0.09 (se =0.007) and $\beta\ year_{TG}$ =-0.003 (se =0.0008) respectively, p-values<0.05), but the decreasing trend was not significantly different between males and females. Conversely, HDL-C showed a modest yearly increase ($\beta\ year_{HDL\text{-}C}$=0.005 (se =0.001), p-value<0.05) for both sexes. However, calendar year at examination did not appear to modify the average age-related trajectories of either lipid in the cohort (i.e. all 3-way year*sex*age-terms interactions were non-significant, p-values >0.05).

The sigmoidal function of age developed by Wineinger et al. 2013 [283] did not fit the lipoprotein profiles in the YFS study sample *(lipoprotein $_{(t)}$ ~ intercept + sin(π/2 * ((2 * age$_{(t)}$ – max(age)/min(age)) *sex)*, and the best non-linear fits were achieved by using a 4th degree polynomial age term for HDL-C profile and 3rd degree polynomial age term LDL-C and triglycerides profiles (i.e. models yielding the lowest AIC and BIC values). Final IGC models assess the effect of sex, wGRSs and their interaction as predictors of the individual variability in lipid growth parameters over the life course. Model selection for the optimal random effects structure revealed that a continuous first order autoregressive correlation structure was needed in each model for the error term, implying that the within-subject correlation between lipid measures drops exponentially with increasing temporal separation (see Appendix 3.10 for model validation plots of the final IGC model). Fig 3.1 shows the predicted average sex-specific lipid trajectories for the participants in the upper and lower

wGRSs quartiles with corresponding 95% confidence intervals, determined from the estimated parameters and SEs of the final IGC models (i.e. prototypical growth curves). Sex-specific prototypical growth curves show that trends in lipoproteins from age 3 to 49 years were different for males and females in the cohort. When stratified by sex, participants from high or low genetic risk groups (upper and lower wGRSs quartiles) showed differences in average levels of each lipoprotein from age 3, but displayed average profiles of globally similar shapes over time (Fig 3.1).

Participants 10 years and younger already had high-risk or close to high-risk average levels of LDL-C (especially for participants in the high genetic risk group, and females in the lowest LDL-C wGRS quartile) as defined by the NCEP pediatric and adolescent cut points [272] (Fig 3.1). The decrease in HDL-C levels in the cohort was noticeable above the age of 30 years and was more pronounced among males, who in early life had higher average HDL-C levels compared with females, independent of their wGRS status. Similarly, triglyceride profiles in the cohort show a sex-dependent divergence over time, with males tending to exhibit higher average triglyceride levels compared with females from their early to mid 20's both in the high and low wGRS groups. Fig 3.1 also suggests the divergence by sex in adulthood is more pronounced in the high risk TG wGRS participants, suggesting males in this group have exacerbated average triglyceride levels from age 25 years onwards.

The output of the wGRS group- and sex-adjusted individual growth curve models corroborate the observations that the genetic effect is already present in childhood for each of the three lipids, with significant time independent effects of polygenic genetic risk score categories on baseline lipoprotein levels (Table 3.2, $\beta s_{\text{high-low}}$ and $\beta s_{\text{high-mid}}$). Indeed, participants in the low and mid LDL-C wGRS group have LDL-C concentrations lowered by 46%, and 23% respectively (p-values <0.0001) as compared to participants in the high LDL-C wGRS. The analysis of HDL-C profiles revealed that participants in the low and mid HDL-C wGRS had average baseline HDL-C levels increased by ~16.8% and ~8% respectively compared with participants in the high risk score group (p-values <0.0005) (Table 3.2). Similarly, participants in the low and mid TG wGRS had average baseline triglyceride levels lowered by ~20% (i.e. log (0.8)) and ~10% (i.e. log (0.9)) respectively compared with participants in the high risk score group (p-values <0.0005).

Table 3.1 Average lipid concentrations in childhood, young adulthood and middle adulthood, across 1980-2011 (all reported in mmol/L), and genetic risk factors (no unit) considered in the longitudinal lipoprotein profile analyses.

		Males	Females
HDL Analysis		(N=1064**)	(N=1244**)
	Average HDL-C*	1.37 (0.36) (N†=9043)	1.51 (0.32) (N†=10540)
	3-15 years	1.58 (0.34) (N†=2649)	1.57 (0.30) (N†=3078)
	18-30 years	1.28 (0.30) (N†=3374)	1.52 (0.33) (N†=3937)
	33-49 years	1.19 (0.29)(N†=3020)	1.42 (0.31) (N†=3525)
	Genetic risk:		
	Average HDL wGRS	32.46 (3.36)	32.62 (3.41)
	High score (wGRS >34.8)	N=253 (25%)	N=324 (25%)
	Mid score (30.1<wGRS≤34.8)	N=541 (50%)	N=613 (50%)
	Low score (wGRS ≤30.1)	N=270 (25%)	N=307 (25%)
LDL Analysis		(N =1121**)	(N = 1314**)
	Average LDL-C*	3.22 (0.86) (N†=9530)	3.17 (0.81) (N†=10834)
	3-15 years	3.12 (0.83) (N†=2781)	3.32 (0.84) (N†=3255)
	18-30 years	3.07 (0.85) (N†=3563)	3.06 (0.80) (N†= 3856)
	33-49 years	3.41 (0.85) (N†=3186)	3.07 (0.74) (N†=3723)
	Genetic risk:		
	Average LDL wGRS	42.1 (6.6*)	41.9 (6.9*)
	High score (wGRS >46.1)	N=278 (25%)	N=332 (25%)
	Mid score (37.5<wGRS ≤46.2)	N=553 (50%)	N=665 (50%)
	Low score (wGRS ≤37.5)	N=290 (25%)	N=317 (25%)

Triglycerides Analysis		(N =1121*)	(N = 1314*)
	Average Triglycerides*	1.17 (0.96) (N†=9513)	1.00 (0.56) (N†=11148)
	3-15 years	0.73 (0.32) (N†=2776)	0.79 (0.34) (N†=3257)
	18-30 years	1.17 (0.69) (N†=3557)	1.06 (0.53) (N†=4163)
	33-49 years	1.56 (1.10) (N†=3180)	1.15 (0.89) (N†=3728)
	Genetic risk:		
	Average TGwGRS	32.71 (15.81)	131.91 (15.72)
	High score (wGRS >142.3)	N=280 (25%)	N=334 (25%)
	Mid score (121.61<wGRS ≤142.3)	N=280 (25%)	N=660 (50%)
	Low score (wGRS ≤121.6)	N=275 (25%)	N=322 (25%)

*Data are sex-specific averages (SD) for lipoprotein concentration and for continuous genetic risks cores (wGRSs) collected for the entire study sample between 1980 and 2011 (the average age of male participants was 24.2 (11.8) years and the average age of female participants was 24.2 (11.8) years over the study period, which was not significantly different). Presented are also average (SD) lipoprotein levels stratified by age group (i.e. childhood (3-15 years), young adulthood (18-27 years), and middle adulthood (30-49 years). The grouping of wGRSs into categories was based on whole-cohort 25th and 75th percentiles (See methods).

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

**Indicates the number of participants included in the longitudinal lipoprotein profile analyses.

† Indicates the number of available measurements for the calculation of each average lipoprotein concentration.

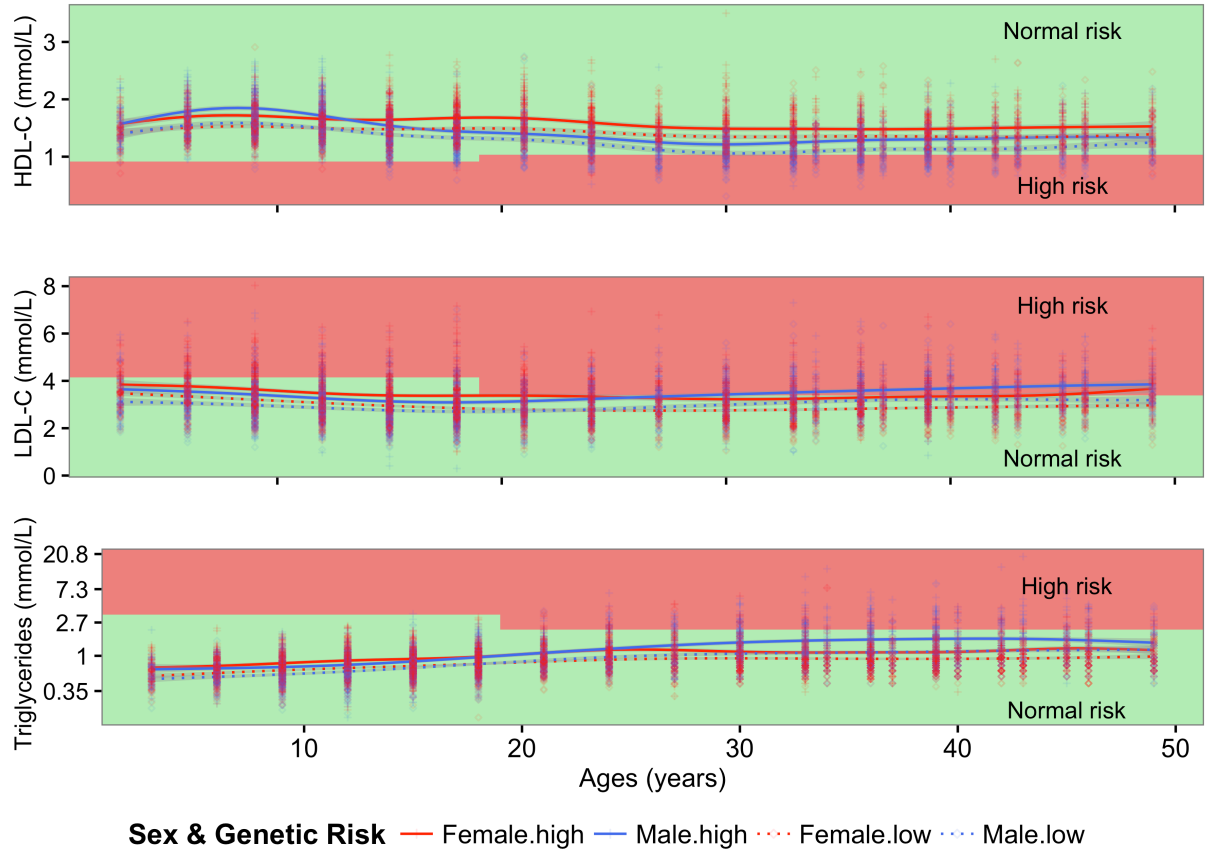

Fig 3.1. Scatterplot of serum lipoprotein longitudinal profiles of participants according to their sex and wGRSs status (High and Low wGRS*) (N=2435, N= 2308 and N=2435 for LDL-C, HDL-C, and triglyceride (TG) profiles respectively).

Solid and dotted lines represent estimated sex-specific average age-related lipid trajectories for participants in high and low genetic risk score, respectively (i.e. prototypical growth curves); blue and red coloured bands around the growth curves represent approximated 95% prediction CI. Overlaid with the prototypical lipid trajectories are the age-specific cut points for lipoprotein status (normal vs. high risk) as defined by the NCEP adolescent and childhood classification [272] and NCEP adult-treatment panel guidelines [273]). The cut points represented in green/red blocks are used to identify those at significantly increased risk of developing atherosclerotic CVD in adulthood. * Mid wGRS risk group are not presented in the figure for the purpose of readability.

Analyses of interaction terms revealed no time-dependent polygenic effects of the 14 risk SNPs on circulating LDL-C, implying that there is no worsening effect of LDL-C levels over time among those belonging to the high genetic risk group compared with those in the low or mid genetic risk group (all linear, quadratic and cubic $\beta_{wGRS *age}$ interactions p-values >0.3). On average at baseline, males had 7% higher LDL-C levels and 15% lower HDL-C levels compared with females (p-values <0.004). Sex appears to be the variable that drives the longitudinal trajectory of LDL-C levels in this cohort ($\beta_{sexMale*f(age)}$=0.019, se=0.0007, p-values <0.001), rather than wGRS group membership (linear, quadratic, and cubic rate of change not significant, Table 3.2).

For HDL-C however, a significant linear and quadratic age-dependent interactions were found between participants belonging to the high and low wGRS group ($\beta_{[wGRS *f(age)]}$=0.022 and $\beta_{[wGRS *f(age-square)]}$=-2.6 x$10^{-04}$) (Table 3.2), suggesting that genetic group membership is a modifier of the HDL-C trajectory. The positive effect of high wGRS on the linear age term implies that, for children in the high wGRS category, the effect of the combined variants leads to an initial increase that is faster by 2.2% per year compared with children in the low wGRS group (i.e. slightly steeper linear increase). Similarly, the negative effect on the quadratic rate of change also indicates that for participants in the high wGRS group, the positive genetic effect on HDL-C levels will decelerate in time slightly slower (by on average 0.026%) than it does in the low GRS group around adolescence. This small difference in HDL-C trajectories between high and low wGRS groups is not easily distinguished in Fig 3.1, as the modifying effect of the variants on the trajectory parameters is relatively mild. These age-dependent interactions were not modified by sex (all 3-way interactions were not significant p>0.05), so that the effect of wGRS categories on HDL-C trajectory parameters were not significantly different in males and females.

For triglycerides, the linear, quadratic, and cubic age-dependent interactions between participants belonging to the high and low wGRS group are not significant in females (Table 3.2). However, for males, genetic risk group membership modified the linear change if triglycerides (3-way interactions between linear age-dependent change rate, sex, and TG wGRS group membership) were significant both for low vs. high genetic risk group (exp$\beta_{[male*wGRS *f(age)]}$=0.96, se=0.003, p-value=0.0009), and for mid vs. high genetic risk group (exp $\beta_{[male*wGRS *f(age)]}$=0.99, se=0.002, p-value=0.01). That is, males belonging to the

high genetic risk group tended to have a linear increase in TG levels by 4% (i.e. log(0.96)) and by 1% (i.e. log(0.99) for participants in the mid genetic risk group. As these effects are also moderate, it does not result in strong divergences in the prototypical triglyceride trajectories (Fig 3.1).

Table 3.2. Time-averaged and time-dependent effects of the categorical combined genetic risk scores (HDL-C , TG and LDL-C wGRSs) on lipoprotein concentrations (mmol/L) from childhood through adulthood. Regression coefficients (βs) are in % change vs. the reference group (i.e. high genetic risk group).

		Main wGRS effects[a]		Time-dependent wGRS effects[b]		
Lipoprotein		**β(se)**	**p-value**	**β(se)**	**p-value**	**Goodness of fit**
HDL-C†	*High vs. Low*	-0.17 (0.01)	0.0001*	L: 0.022 (0.0015)	0.005*	Marginal R^2: 0.21
				Q: $-2.6x10^{-4}$ ($1.1x10^{-3}$)	0.02*	Conditional R^2:0.72
				C: $2.5x10^{-6}$ (3.4x10-^{6})	0.47	
				4^{th}: 4.0x10-^{7} ($2.4^{-}x10^{-7}$)	0.09	
	High vs. Mid	-0.08 (0.012)	$1.0x10^{-4}$*	L: 0.0014 (0.0009)	0.11	
				Q:- 1.0x10-^{4} ($9.0x10^{-5}$)	0.28	
				C: $2.5x10^{-6}$ ($5.1x10^{-6}$)	0.40	
				4^{th}: 1.0x10-^{7} ($2.1x10^{-7}$)	0.67	
LDL-C††	*High vs. Low*	-0.46 (0.04)	$1.0x10^{-4}$*	L: - $7.0x10^{-3}$ (0.002)	0.72	Marginal R^2: 0.11
				Q: $2.8x10^{-5}$ ($9.5x10^{-7}$)	0.76	Conditional R^2: 0.71
				C: $3.0x10^{-6}$ ($6.6x10^{-7}$)	0.71	
	High vs. Mid	-0.23 (0.03)	$1.0x10^{-4}$*	L: $6.1x10^{-2}$ ($1.9x10^{-2}$)	0.52	
				Q: $6.3x10^{-5}$ ($6.1x10^{-6}$)	0.45	
				C: $1.3x10^{-9}$ ($1.0x10^{-9}$)	0.94	

Triglycerides †††	*High vs. Low*	0.80 (0.025)	0.0001*	L: 0.995 (0.001)	0.11	Marginal R^2: 0.18
				Q: 0.99 ($6.8x10^{-5}$)	0.08	Conditional R^2:0.68
				C: 1.00 ($4.9x10^{-7}$)	0.37	
	High vs. Mid	0.90 (0.022)	$1.0x10^{-4}$*	L: 0.999 (0.001)	0.15	
				Q:- 0.997 ($5.8x10^{-5}$)	0.09	

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *High/Mid/ Low,* categorical genetic risk score groups (for either lipoprotein trait); *L, Q, C and 4th*, Linear/quadratic/cubic/ and quartic rate of change (in either lipoprotein concentration as a function of age).

† / †† / ††† wGRS effects refer to the combined effect of the 38-, 14- and 24 SNPs associated respectively to HDL-C, LDL-C and fasting triglycerides levels (see methods section 3.3).

* Indicates that the estimated regression parameter is significant at the 0.05 significance level.

[a,b] For ease of interpretation of the estimates of main and time-dependent effects of wGRSs, all age terms were centered around youngest childhood age at baseline (1980) in the cohort (3 years old) prior regression analysis. For triglycerides, regressions coefficients βs of main and time-dependent effects were exponentiated so they are presented in the original scale for ease of interpretation.

When using the wGRSs values as continuous predictors, the final IGC models for the age-related trajectories of LDL-C, HDL-C and triglycerides profiles achieved a conditional R^2 of 0.64, 0.68 and 0.61 respectively, with the fixed predictors (age, sex, and continuous wGRS) jointly accounting for 12%, 27% and 19% of deviance respectively in each model. Consistent with the categorical analyses, the time-averaged genetic effect of the combined genetic risk score on lipoprotein profiles was significant ($p<0.0001$) for all traits (Table 3.3). The polygenic effect size was stronger for LDL-C associated risk SNPs, with LDL-C wGRS increasing LDL-C levels by 18.2% per SD increase in score (as compared to a 6.8% increase in HDL-C levels per SD increase in wHDL-C GRS). Additionally, wHDL-C GRS was a significant predictor of the linear, quadratic and quartic changes in HDL-C levels over time (p-values <0.01, Table 3.3), implying that the slope of the genetic risk score variable on HDL-C concentration changes as participants age from childhood through adulthood. These time-dependent interactions are best visualised by computing and plotting the marginal effect of the combined genetic risk score on HDL-C levels (Fig 3.2 A). The downward trends of the slopes of the continuous risk score on HDL-C level with age for both males and females, suggests that the association between HDL-C levels and of the 38 HDL-C risk SNPs gets weaker as participants aged in this population, although the combined genetic effect of these loci on HDL-C was consistently stronger among females.

For triglycerides, continuous analyses revealed that while on average, TG wGRS did not modify the linear, quadratic or cubic change in triglyceride level, but when clustering participant by sex, TG wGRS effect on the linear change rate in triglyceride levels became significant for males (three way interaction $\exp \beta_{wTGgrs24*sexMale*f(age)}=1.0038$, se=0.0011, p-value=0.001). This difference between sexes can also be visualised by plotting the marginal effect of the TG combined genetic risk score on triglyceride levels (Fig. 2.2 B). For females, a 1-sd increase in risk score will result in 9% higher serum triglyceride on average (regardless of their age; log (1.1)=0.09). For males, the effect size of TG genetic risk score is age-dependent and lower in childhood than for females, it increases linearly to become larger in females from age 25 onwards, reaching 13% at age 49 years (log(1.14)=0.13).

Table 3.3. Time-averaged and time-dependent effects of the continuous combined genetic risk scores (HDL-C , LDL-C and TG wGRSs) on lipoprotein concentrations (in mmol/L) from childhood through adulthood.

	Main wGRS effects[a]		Time-dependent wGRS effects[b]		
Lipoprotein	**β(se)****	**p-value**	**β(se)****	**p-value**	**Goodness of fit**
HDL-C †	0.0064(0.002)	$1.0x10^{-4}$*	L : $-6.1 x10^{-3}$ ($2.0 x10^{-4}$)	0.003 *	Marginal R^2: 0.27
			Q: $2.0 x10^{-5}$ ($4.1 x10^{-6}$)	0.014*	Conditional R^2: 0.68
			C: $4.0 x10^{-6}$ ($1.2 x10^{-7}$)	0.07	
			4^{th}: $-1.0 x10^{-7}$ ($7.8 x10^{-9}$)	0.05	
LDL-C††	0.182 (0.001)	0.0003*	L: $2.7x10^{-4}$ ($1.2 x10^{-3}$)	0.85	Marginal $R^{2:}$ 0.13
			Q: $-9.5 x10^{-5}$ ($1.5x10^{-4}$)	0.44	Conditional R^2: 0.64
			C: $3.0 x10^{-6}$ ($2.6 x10^{-6}$)	0.23	
Triglycerides†††	1.094 (0.008)	$1.0x10^{-4}$*	L : 1.0 ($9.8 x10^{-4}$)	0.47	Marginal R^2: 0.20
			Q: 1.0($8.0 x10^{-5}$)	0.31	Conditional R^2: 0.65
			C: 0.99 ($1.7 x10^{-7}$)	0.20	

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *L, Q, C and 4th*, Linear/quadratic/cubic/ and quartic rate of change (in either lipoprotein concentration as a function of age).
† / †† / ††† wGRS effects refer to the combined effect of the 38-, 14- and 24 SNPs associated respectively to HDL-C, LDL-C and fasting triglycerides levels (see methods).
* Indicates that the estimated regression parameter is significant at the 0.05 significance level.
[a,b] For ease of interpretation of the estimates of main and time-dependent effects of wGRSs, all age terms were centered around youngest childhood age at baseline (1980) in the cohort (3 years old) prior regression analysis.
**Regression coefficient βs are in mmol/L per 1-sd change in wGRS for HDL-C and LDL-C. For triglycerides, regressions coefficients βs of main and time-dependent effects were exponentiated so they are presented in the original scale for ease of interpretation.

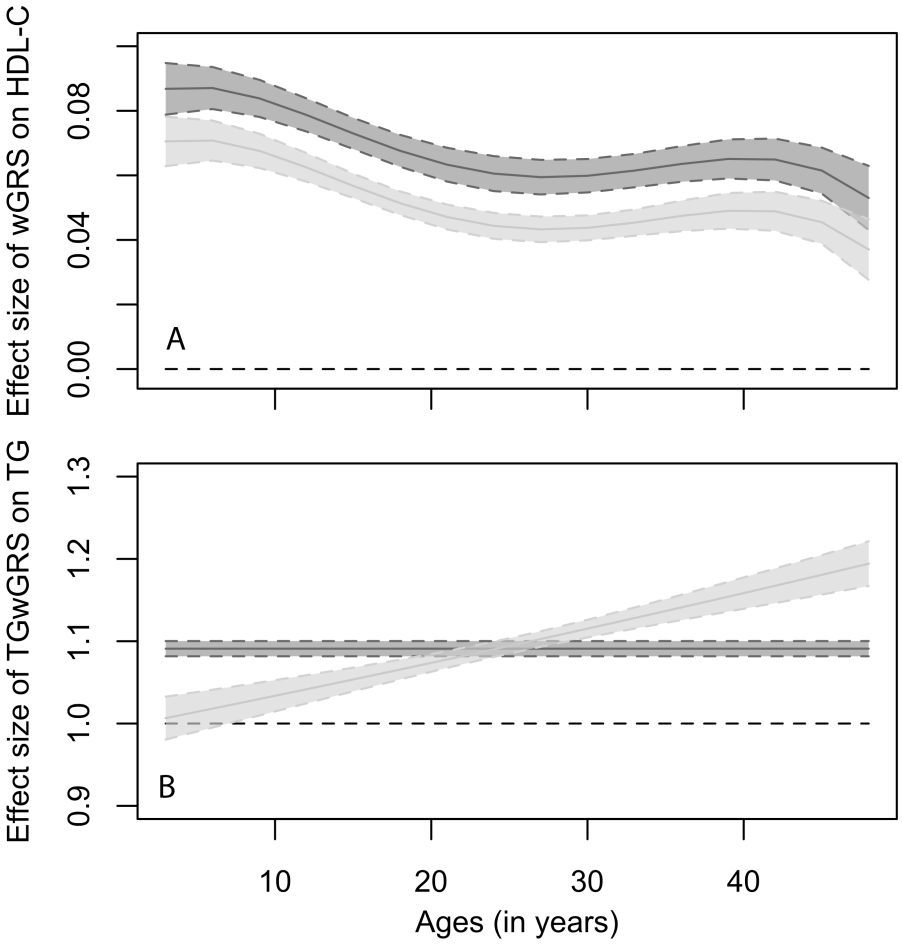

Fig 3.2. Sex-specific marginal effect* and 95% CI of (A) combined continuous HDL-C wGRS on HDL-C levels (effect size expressed in mmol/L lipoprotein level change per 1-sd change in wGRSs); and (B) combined continuous TG wGRS on fasting triglyceride levels (effect size expressed in odds ratio lipoprotein level change per 1-sd change in wGRSs).

Colour code: dark grey; females, light grey; males.
*Plotted marginal effect includes the significant linear slope, quadratic and quartic rates of change (cubic trajectory parameter not significant in the final model); Horizontal black dashed line shows where the slopes are not significantly different from zero).

3.4.2 Age-stratified linear regression

Cross sectional analyses confirmed that combined wGRSs were significantly associated with the three lipoproteins at all ages for both sexes (Fig 3.3, all p-values <0.05). Confirming that the joint effect of the 14 risk SNPs on LDL-C levels is consistent across time, the fitting of a LOESS regression lines to the ages- and sex-specific regression parameters did not reveal any striking trend over time (Fig. 3.3, upper panel). It may be

that the variations in effect sizes between different age-groups were mostly attributable to differences in sample sizes and differences in the number of birth cohorts used in the regression analysis (only one birth cohort for the 3 year old age-group, against a mix of up to 5 birth cohorts for the 15 and 18 year old group). However, the age- and sex- stratified analysis of the association between HDL-C levels and HDL-C genetic risk, confirmed that the effect size of HDL-C wGRS on HDL-C decreased almost by half, on-average, for age-groups >30 years in males and females. For triglycerides, the results of the age-stratified regression analysis are consistent with the results of the individual growth curve analyses, showing very stable effect estimates for females throughout age groups, and a clear upward trend in effect size estimates for males. Sex-specific mean effect sizes for categorical wGRS at chosen ages (i.e. 3, 15 24 and 45/46 years) are consistent with the results of the sex-and stratified cross sectional analyses of the continuous risk scores (Appendix 3.7).

3.4.3 Evidence for polygenic gene-lifestyle interaction on adult lipoprotein levels

Change in BMI z-score between adulthood and childhood (Δ BMI) was highly predictive of each adult lipoprotein levels independently of the wGRSs in multivariable models adjusted for sex and age at baseline and follow-up (Appendix 3.3). However, the LR tests revealed no evidence for gene-lifestyle interactions (Δ BMI *wGRSs) on adult lipoprotein concentrations, as the combined effect of candidate genes on adult lipoprotein levels does not appear to be modulated by the trajectory of BMI from childhood in this cohort (χ^2(df=1)=0.24 p=0.62; χ^2(df=1)=2.04, p-value=0.15 and χ^2(df=1)=1.17 p-value=0.11 for HDL-C, LDL-C, and triglycerides respectively).

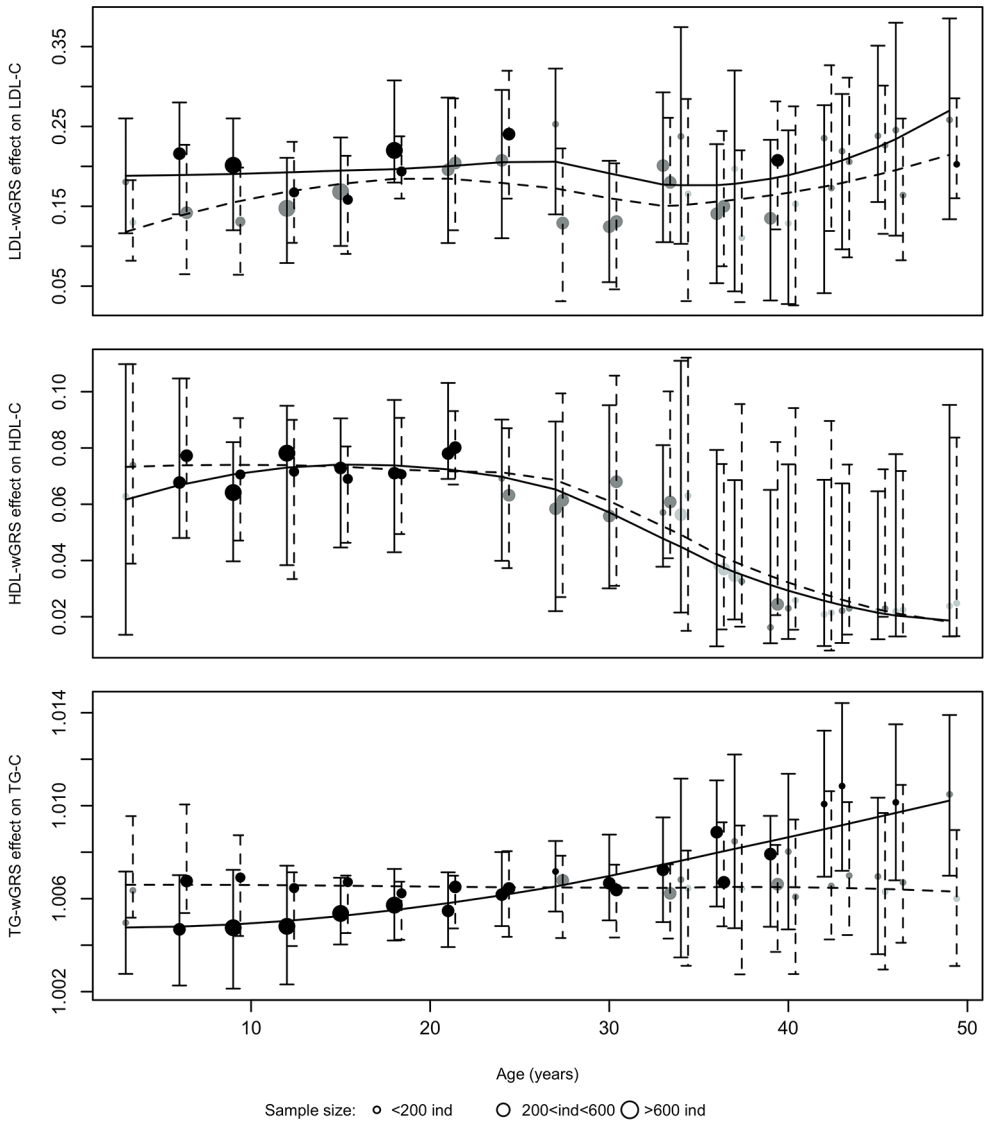

Fig 3.3. Age- and sex- stratified estimated effects of LDL-C wGRS (upper panel), HDL-C wGRS (middle panel) and TG wGRS (lower panel) on LDL-C, HDL-C and triglycerides levels (mmol/L) respectively with colour coded significance levels and studentised bootstrapped non-parametric 95% CI .

For each age, the continuous error bars correspond to males and the dashed error bars directly next to them correspond to female models. Effect sizes are in mmol/L change per 1-sd change in wGRS for LDL-C and HDL-C and in odds ratio lipoprotein level change per 1-sd change in wGRS for triglycerides. Point sizes of the beta estimates reflect sample size (number of participants included in each age- and sex-specific regression analysis) Parameter estimates significance : Light grey, $0.05<\text{p-val}<0.01$; Dark grey, $1.x10^{-3}<\text{p-val}<1.X10^{-6}$; Black, $\text{p-val}\leq 1.X10^{-6}$. Black lines: smooth trend curves fitted by LOESS (Locally weighted non-parametric regression) to help visualise trends in the cross-sectional associations.

3.5 Discussion

This is the first study to investigate the combined effect of dyslipidaemia–predisposing variants on longitudinal blood lipoprotein profiles from childhood to adulthood. Rather than considering individual-validated risk lipid-SNPs alone, usually characterised by weak to moderate effect sizes in large lipid GWASs, the approach used here weighted genetic risk scores that combine multiple loci identified in meta-analyses of large lipid GWAS. Collectively, our findings suggest that genetic factors influence age-specific lipoprotein values and developmental trajectories already from the age of 3 years.

The use of polygenic risk scores has become increasingly popular in recent years for the purpose of genetic prediction of a number of quantitative traits, with the increasing recognition that a substantial part of heritability comes from many susceptibility markers individually characterised by low predictive power [261]. For circulating lipids in particular, when combined, multiple common genetic variants with small effects on circulating lipid levels, were reported to be highly predictive of individual trait measures [263] and showed association with subclinical and clinical cardiovascular outcomes [262]. Despite these promising associations between SNPs (or genetic risk scores) and lipoproteins, susceptibility alleles are often identified from cross-sectional adult GWASs, and it remains unclear whether the within-individual level of genetic risk carried by these variants is stable through life or changes with age. This uncertainty greatly impedes the assessment of the abilities of individual SNPs (or polygenic GWAS-derived risk score) for prediction of quantitative traits collected across the life course and their clinical usefulness in the primary prevention of dyslipidemia.

Independent of participant age-related lipid trends, significant decreasing secular linear trends in LDL-C and triglycerides across the 31 follow-up years were found, which is consistent with what has been reported in previous studies [284]. These trends may be partially due to a wider use of statins or improvement in diet in the last decades[252].

Multilevel individual growth curve (IGC) analysis revealed an expected difference in lipoprotein profiles from childhood to adulthood between males and females, and although baseline HDL-C, LDL-C and TG levels were not significantly different between sexes (at age 3 years), the trajectories of lipid levels from childhood to adulthood were largely sex-

driven. A time-averaged association between lipid wGRSs and each of the three serum lipid levels was observed, suggesting that composite genetic risk scores were robust predictors of average lipoprotein levels, as well as predictors of lipoprotein levels in childhood (from age 3 years). The time-averaged effect of the 14 risk alleles on LDL-C levels was stronger on average than the effect of the 38 risk SNPs on HDL-C levels, suggesting that the genetic predisposition to high serum LDL-C is stronger compared with a predisposition to low HDL-C levels and high triglycerides levels. The LDL-C wGRSs did not modify the linear, quadratic, or cubic trajectory of lipoprotein level, so that the combined effect of the 14 candidate SNPs is not only present in young childhood, but also consistent across a person's life course as he/she ages (from age 3 to 49 years). Categorical analyses revealed the 38 risk SNPs modified the linear and quadratic component of HDL-C trajectory over time, although the effect sizes of the age-dependent terms were small, such that it does not translate to clearly divergent profiles between high- and low-risk participants. However, when plotting the estimated marginal effect of HDL-C wGRS over time (within-individual), for a given male participant the magnitude of the effect of the combined genetic risk score on his HDL-C levels will attenuate with age. This is also confirmed by the cross sectional age-stratified analyses, where childhood HDL-C levels were strongly related to genetics, but where the main effect of HDL-C wGRS diminished by half in age-groups over 30 years old. Although significant at all ages, the collective effect of the HDL-C risk score on HDL-C levels becomes weaker as participants age, with environmental, lifestyle and behavioural factors such as diet, smoking and physical activity, potentially becoming more important determinants of adult circulating HDL-C.

For triglycerides, it was found that the combined effect of the 24 risk SNPs on triglyceride levels appears consistent over time for females from childhood through adulthood. For males however, both the cross sectional regression and IGC analyses suggest that the association between TGwGRS and fasting triglycerides levels increases linearly with age, becoming stronger on average than in females from around 25 years of age. These findings suggest that in adulthood, males at risk may respond less efficiently than females to lifestyle interventions targeting the reduction of fasting triglyceride levels

For lipids, it is largely unknown how a conventional age-varying 'lifestyle-related' risk factor such as adiposity modifies the genetic risk of developing abnormal lipid over the life

course. This is a particularly relevant topic as identifying people whose risk is amplified by a combination of genetic and behavioral factors might facilitate interventions to prevent or delay the onset of cardiometabolic diseases. Change in BMI z-score (Δ BMIi), a proxy retrospective indicator of 'adiposity trajectory', was computed for each participant to summarize both the directionality and magnitude of change in their weight status-related lifestyle factors over their life course relative to the average change in the cohort. In these analyses, the change in BMI z-score from childhood to adulthood predicted adult lipoprotein levels in 2011. This observation is consistent with the fact that adult BMI is important for many adult metabolic factors including lipids and that associations between lipids and BMI generally strengthened with age [167]. However, the effect of BMI z-score change since childhood on adult concentration is not modified by the wGRS for any of the lipoproteins, signifying that the detrimental consequences of an above average change in BMI since childhood on circulating lipoprotein levels does not appear to be exacerbated in adults that are genetically predisposed to high-risk lipoprotein profiles. However, this analysis is preliminary, and although it considers the overall direction of change in BMI z-score between childhood and adulthood, it does not fully account for the age-varying nature of BMI as a confounder and how it might interact with wGRS at different age-or developmental stages (childhood, adolescence, young adulthood). A study looking at the importance of the age at which obesity developed in associations between adult lipids and weight status, showed that although obese adults had adverse levels of lipoproteins, these levels did not vary with childhood weight status or with the age at the onset of obesity[285]. However, it remains unknown if specific age of obesity onset modifies the effect of a genetic predisposition to averse lipoprotein levels, and whether primary prevention measures could be improved by specifically aiming at resolving obesity before a certain 'critical' age-window in individuals genetically at-risk to develop dyslipidemia, as has been shown for apparently healthy individuals [33].

3.6 Strengths and limitations

The present work has a number of strengths and limitations. Strengths included the study of a large, randomly selected cohort of men and women followed up at eight occasions over the course of 30 years since early childhood. The extensive longitudinal lipoprotein phenotypic and genotypic data offered a rare opportunity for a refined analysis of the

association between genetic risk and serum lipoprotein trajectories. The hierarchical mixed effect IGC modeling approach allowed the comprehensive modeling of between-individual changes in within individual outcome trajectories. The multivariable models of adult lipoprotein allowed estimating the ability of the polygenic GWAS-derived lipoprotein risk scores to predict adult lipoprotein concentration over other conventional childhood risk factors.

Limitations were also present. Because the YFS cohort is of European descent, the results are only generalisable to individuals with a similar ancestry. Loss to follow-up from the original cohort more often occurs for males, therefore the sex-specific time-averaged and time-dependent effect of multi-loci risk scores might be slightly biased. We also suggest that to validate causal inference, these findings should be tested for potential confounding effects of other variables such as additional adiposity indicators, which are known to correlate highly with lipoproteins and vary over time. However, additional adiposity phenotypes have not been collected at each study wave in the YFS study.

3.7 Conclusion

This study demonstrates the significance of GWAS-derived genetic risk scores as predictors of lipoprotein levels at all ages. Additionally, for the first time, an age-dependent effect of the 38 HDL-C risk SNPs is shown on HDL-C and of the 25 TG risk SNPs on TG levels was found among males, suggesting that the genetically-determined effects on these lipoproteins tends to change as a person ages. These findings are clinically significant, as they may help refine interventions targeted at the prevention of worsening of lipid profiles as people age. Because this study focused on modeling individual lipid profiles across the life course, it did not consider how lipid levels of each participant in adulthood translate in terms of dyslipidemia status. Further studies would be needed to characterise how this polygenic effect translates in terms of disease status prediction from childhood to adulthood, especially to investigate whether polygenic risk scores can help improve the prediction of adult disease beyond traditionally used clinical factors, such as lipid levels in mid-childhood.

KEY POINTS

- This chapter investigates for the first time the combined effect of dyslipidaemia–predisposing common genetic variants on blood lipid profiles from childhood to adulthood in the YFS cohort.
- For LDL-C, HDL-C, and triglycerides, the polygenic effect of GWAS-identified risk alleles was present from early childhood (from age 3 years).
- The strongest time-averaged polygenic effect size was observed for the LDL-C associated variants suggesting that the multigenetic predisposition to high serum LDL-C at all ages is stronger compared with a predisposition to low HDL-C and high triglycerides levels.
- The polygenic effect on LDL-C levels remained and consistent across the life course as YFS participants aged.
- The multigenic effect on HDL-C levels became weaker as participants aged, with environmental, lifestyle and behavioral factors such as diet, smoking, physical activity, potentially becoming more important determinants of HDL-C levels in mid-adulthood.
- The detrimental effect of the 24 risk variants on triglyceride levels increased linearly with age for males, suggesting that from mid-adulthood onwards, males at risk may respond less efficiently to lifestyle interventions targeting the reduction of fasting triglyceride levels.
- This chapter demonstrate how IGC modeling, a person-based approach to trajectory analyses can be used to investigate associations between longitudinal response profiles of complex shapes and important individual cardiometabolic risk marker, such as genetic factors.

Box 1. *Summary of key points from Chapter 3: The combined effect of common genetic risk variants on childhood to adulthood trajectories of circulating lipoproteins in the YFS*

4. BAYESIAN HIERARCHICAL PIECEWISE REGRESSION MODELS: A TOOL TO DETECT TRAJECTORY DIVERGENCE BETWEEN GROUPS IN LONG-TERM OBSERVATIONAL STUDIES

4.1 Preface

In this chapter, the aim was to demonstrate how linear-linear segmented regression model can be used at level-1 instead of the commonly used higher-order polynomial within-person submodel, to formulate a MLM growth curve model able to compare longitudinal change in non-linear continuous responses between distinct developmental periods. Based on this model formulation, a sequential modeling approach was used to allow the estimation of the point in time (or the age) at which two groups of individuals go onto separate curvilinear trajectories. The proposed Bayesian Hierarchical Piecewise Regression approach (BHPR) is suitable to investigate mechanisms of trajectory divergence between groups, allows avoiding some of the limitations of higher order polynomial growth models, and yields parameter estimates that are easily interpretable and that map onto what is known of the development of BMI from childhood to adulthood. The approach consists of fitting a series of growth curve models where group-differences in the conditional means of the growth parameters are sequentially introduced at level-2. These models are estimated in a fully Bayesian framework since not only the focus is to estimate the points at which the groups of interest change growth patterns, but also to allow for subject-to subject variability in these change points (i.e. ages at which they change growth pattern). This novel approach is illustrated by applying the developed methodology to identify age at divergence in the BMI trajectory of YFS participants who go on to develop adult T2DM as opposed to participants who remain healthy in adulthood. The clinical interest of this method is that it could help identify critical windows to intervene to normalise modifiable risk factors for later cardiometabolic outcomes. Additionally, this approach can be used to test and adjust for potential between-cohort heterogeneity in the response variable that may be present in multi-cohort designs. Through simulation, it is demonstrated that this method is superior to a commonly used approach to determine age at divergence between groups, that is, the pairwise

comparison of predictions form a categorical mixed models between-group (i.e. least-square means) at each time point.

In summary, in line with general aim 2 and 3 of this thesis, this chapter has developed a novel GCM approach which allows to determine between-group trajectory divergence in long-term observations studies, and illustrates the use and relevance of the proposed approach by identifying the age at which YFS participant who develop T2DM go on to different BMI paths compared to those participant wo do not develop T2DM.

4.2 Introduction

Child to adult trajectories of health markers are likely to have implications for the risk of chronic diseases in later life, such as obesity, T2DM and CVD; it is therefore important to understand their development throughout the life course [286-288]. Observational studies that follow the same study participants over long periods of time that span from early childhood to adulthood are especially suited to studying adult-onset disorders such as cardiometabolic disease, since they allow characterising the development of normal vs. pathological processes over time. A goal of such studies is often to determine how a number of individual characteristics, modifiable risk factors profiles, their interactions, and normal aging may impact the onset and progression of disease over time, in order to identify time periods of divergence in these factors. [28, 62, 285].

Depending on the condition being studied, longitudinal risk factors of interest range from biological markers (e.g. blood lipids, glucose, inflammatory markers) for which there is no a priori or expected systematic pattern of change across the life-span, to anthropomorphic growth characteristics (e.g. height, weight, BMI, and adiposity), for which trajectories often consist of several well studied and identified consecutive developmental periods. In particular, it is now recognised that human growth and development at different life-stages are associated with adult chronic disease [286, 289] with increased sex- and age-adjusted BMI, playing an important role in the development of conditions such as T2DM[290-292], CVD [49, 144, 285] and some cancers [293].

A key statistical issue in these studies is often to determine if risk factor levels vary over time between and within-groups of participants, and whether different groups are changing in a

similar or different fashion over time [84, 115]. Depending on the study, the stratification of participants into groups can relate to participants' characteristics or exposure (smoking status, sex), intervention arm (i.e. control vs. medication), or the grouping factor could be a later health outcome (e.g. disease status in mid-adulthood). When participants are grouped according to a distal dichotomous health outcome, longitudinal data provide the foundation to understand pathways to deleterious risk factor profiles, which may help inform the timing of public health intervention to prevent or delay adverse health outcomes in adulthood [28, 62, 285].

When it is established that groups of interest start with similar initial outcome levels, but do not change similarly over time, it is often of interest to determine the point in time or age at which groups begin to diverge in their trajectories [69, 143, 187, 194, 294]. Being able to determine how and when the change manifests between groups of participants is important, since it can help pinpoint periods in the life course that are critical in the development of abnormal risk factor profiles [295]. However, there is little methodological guidance in the literature on statistical techniques to achieve this, and several studies have noted a lack of relevant methods to investigate trajectory divergence between groups [161, 194, 295].

A common attempt is to fit a mixed model with time (or age) treated as categorical variable (i.e. non time-ordered /ordinated [5]) to retrieve linear predictions at each age for each group of interest from this model (i.e. means of least squares predictions, sometimes called LS-means [296-298]), and to test for a group difference in these predictions using a number of contrasts (i.e. post-hoc pairwise comparisons). In this case, the age at which the difference between-groups emerge is often the age at which a significant between-group difference materializes in the LS-means [2, 5, 91]. Mixed models that assume an unstructured mean response by treating age or time as categorical variables are attractive because they make no assumption about the shape of the mean trajectory of the outcome over time. Several studies have used this approach to determine at "what times the groups means are different" (e.g. between-subject effect or post-hoc pairwise group comparison, if there are more than two groups), or "at what times the means differ" within each group (within-subject effect testing) [2, 109, 299]. However, even when adjustments are applied for multiple tests [2, 64, 89, 90], many authors advise against the unrestricted use of multiple comparisons among marginal means due to well-documented multiple testing issues, especially the increase in false positive rate as the number of hypothesis tests increases [94, 95, 299]. Mixed models that assume an unstructured mean response by treating age or time as categorical

variables tend to be over parameterised and may be inefficient at detecting main effects [300]. Although pairwise mean comparisons are often only meaningful among groups at the same measurement time point, or across time points within the same group, few software offer custom hypothesis testing and most tend to compute and display all possible pairwise comparisons (i.e. differences between LS-means) by default. This leads to unnecessarily conservative results, since the multiplicity adjustment gets more severe as the number of comparisons involved increases [2, 86, 301, 302]. Beyond these issues, another crucial disadvantage of this approach, is that it only tests for the difference in means between groups at each time point and does not provide any information on subject-specific response evolution in time [75, 84], so that the age (or point in time) at which the group difference manifests is ultimately a question of sample size and statistical power.

In contrast, continuous time models such as individual-based trajectory modeling methods, including mixed effect [115], hierarchical [67], multilevel [66], and the closely related structural equation and Latent Growth Curve models [129], have become invaluable tools to understand the natural history of health outcomes as well as risk factor/determinant trajectories [23, 28, 97, 99]. They have well-documented advantages over traditional approaches to repeated-measure data analysis in terms of flexibility, with their main advantage being that they allow summarizing each participant's outcome trajectory with a few trajectory parameters [75, 303]. In addition, they permit the explicitly modeling of *inter-individual* differences in *intra-individual* change, permitting inference regarding the average response trajectory over time and how this evolution may vary with participant characteristics (i.e. participant-level predictors) [18, 19, 21, 130].

Despite their flexibility, these models are not often used to analyse sparse long-term observational data since accelerated longitudinal designs [28, 161, 175] and non-linear response over time [70, 99, 158, 159] both introduce significant complexity into the growth curve modeling approach [22, 65, 137, 138]. Indeed, being able to represent non-linear patterns with a relatively small number of measurement occasions per participants (often <10 time points) and be specific about where between-participant heterogeneity appears in those patterns is a statistical challenge.

Many applications often rely on higher order time (or age) polynomials or latent basis coefficients [27, 28, 81, 99, 103, 194], with strengths and limitations of these approaches well-described [1, 82, 173, 174, 303]. In the context of this study, the polynomial parameterisation of the growth model

does not specifically yield an age or point in time when the growth pattern is changing within- and between-groups. Alternatively, piecewise models, also known as linear splines or broken stick models, can be used to break up a non-linear or curvilinear growth trajectory into several separate linear components [191]. They are particularly useful to compare growth rates in different periods over time if the functional form of the response is characterised by different phases of development, or if there is a shift in the outcome trajectory at some point in the event window (i.e. an acceleration or a deceleration in the response change rate from one point in time (or age)) [24, 83, 180, 181, 184, 185]. Piecewise linear trajectory models have been used to model 'multiphase' developmental processes primarily with 'fixed' transition points in a variety of applications in the frequentist multilevel [23, 67, 184, 203] and structural equation modeling framework [129, 148]. Bayesian applications of these processes are often referred to as 'random change point model' where the position of individual breakpoints is also estimated, allowing for between-person variability in the transition points [125, 154, 190, 192, 193, 196, 198-201]. Univariate Bayesian change point models have notably been used to model various clinical endpoints such as cognitive function decline in dementia sufferers [153, 154], progression of viral loads in HIV patients in response to treatments [196, 198], and change in marker levels of prostate and ovarian cancers [193].

Few studies have, however, investigated the inclusion of categorical covariates or grouping variables as level-2 predictors of the variability in the change point, and the random Bayesian change point model has not been formulated to test specifically for the existence of a 'trajectory divergence' between two (or more) known groups of participants that have longitudinal responses characterised by distinct developmental phases.

In this chapter, the use of Bayesian hierarchical piecewise regression modeling to detect trajectory divergence between groups of participants is illustrated using longitudinal BMI data from the YFS Study. Previously published work on these data, based on categorical mixed modeling, suggested that BMI levels became statistically different between those who develop T2DM in adulthood and those who did not from the age of 15 years [292]. These data are reanalysed to demonstrate how the Bayesian method can be used to: (1) model the BMI profiles to better understand the natural history of the BMI trajectories in those who do and do not develop T2DM in adulthood while controlling for potential cohort effects; and (2) obtain a refined estimate and confidence interval of the age at which the two groups begin to diverge

from one another, translating into significantly different BMI from a certain age onwards. In this application, we postulate that the proposed methodology for the analysis of longitudinal BMI measurements taken periodically (~ 3 yearly) from childhood across adolescence and young adulthood could help identify those at increased risk of developing adult T2DM based on their individual BMI developmental trajectories from childhood to adulthood, potentially increasing the prediction of adult T2DM compared to approaches considering only one BMI measurement in childhood. In addition, a series of short simulations are conducted to illustrate the difference in the estimates of age at divergence when using the traditional approach (i.e. pairwise comparisons of marginal means from a categorical mixed model) vs. the proposed trajectory modeling approach.

The Methods present the proposed statistical approach to formally test for and characterise the trajectory divergence mechanisms between 2 or more groups of participants, and describe the BMI and T2DM data from the longitudinal YFS as a motivating example to illustrate this novel modeling approach. The Results investigate the heterogeneity in BMI development between YFS participants who did and did not develop T2DM using the Bayesian Hierarchical Piecewise Regression method to determine the age at which the divergence occurred between-groups while accounting for potential birth cohort effects, and present the results of a series of short simulations to illustrate the difference in the estimates of age at divergence when using the traditional approach (i.e. pairwise comparisons of least-square means estimated from a categorical mixed model) vs. the proposed trajectory modeling approach. We then discuss the proposed approach.

4.3 Methods

4.3.1 No-covariate model

To accommodate the curvilinear developmental pattern in an individual continuous response over time while providing and adequate representation of its developmental theory, a linear-linear piecewise regression model was considered as the functional form of change in the trajectory model. This *change point* model consists of two joining linear segments and involves four individual specific random effects, representing two known natural developmental phases of the outcome of function of age (or time), The change point (CP) represents the age (or time)

at which the transition to a different growth rate occurs. Throughout, "age" will be used rather than "time" as the relevant time variable. The following unconditional (no covariates) multilevel model was considered:

Level-1 model: (4.1)

$$Reponse_{ij} = b_{0i} + b_{1i}age_{ij}.(1 - u_{CP_i}(age_{ij})) + b_{2i}(age_{ij} - CP_i).u_{CP_i}(age_{ij}) + \varepsilon_{ij}$$

Level-2 model: (4.2)

$$b_{0i} = \beta_{00} + v_{0i}$$
$$b_{1i} = \beta_{10} + v_{1i}$$
$$b_{2i} = \beta_{20} + v_{2i}$$
$$CP_i = CP + v_{CPi}$$

$$\begin{pmatrix} v_{0i} \\ v_{1i} \\ v_{2i} \\ v_{CPi} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma^2_{v0} & \dots & \cdots & \dots \\ \sigma_{v01} & \sigma^2_{v1} & \dots & \dots \\ \sigma_{v02} & \sigma_{v12} & \sigma^2_{v2} & \dots \\ \sigma_{v0CP} & \sigma_{v1CP} & \sigma_{v2CP} & \sigma^2_{CP} \end{pmatrix}\right] \quad (4.3)$$

Where, at age *j* for participant *i*, $Response_{ij}$ is the repeated continuous outcome measures, and age_{ij} is the corresponding time related variables, centered around its grand mean. $u_{CP_i}(age_{ij})$ is a unit heavyside step function where $u_{CP_i}(age_{ij})$=1 if $age_{ij} \geq CP_i$ and $u_{CP_i}(age_{ij}) = 0$ if $age_{ij} < CP_i$. The random trajectory parameters b_{0i}, b_{1i} and b_{2i} correspond to the individual intercept, the slope before and slope after the person-specific change point CP_i, respectively. For each person *i*, b_{0i}, controls the individual baseline level (or initial status) for the outcome *Y* and its interpretation depends on the centering of the age- or time-variable (e.g. if age is centered around 25 year, b_{0i} will be the expected participant-level response at 25 years of age given they are in the first phase of growth b_{1i}). b_{1i}, b_{2i} and CP_i, are the expected linear increase per year of age in the first phase of growth, the expected linear rate of increase after the change point, and age at which the linear perturbation to the initial trend occurs, respectively. ε_{ij} is the level-1 residual (i.e. random within-person error for person *i* at age *j* and is independent and normally distributed (i.e. $\varepsilon_{ij} \sim iid\ N(0, \sigma^2_e)$, and v_{0i}, v_{1i}, v_{2i} and v_{CPi} are the level-2 random

effects multivariate normally distributed with zero mean and variances σ_{v0}^2 , σ_{v1}^2 , σ_{v2}^2 and σ_{CP}^2 respectively and full covariance matrix shown in 1.3 . β_{00}, β_{10}, β_{20} and *CP* are the fixed effects (i.e. population average of each trajectory parameter). In this model, the level-1 residual variance σ_e^2 can be interpreted as the deviations around an individual's trajectory and level-2 residuals as between-participant variability in the overall intercept (σ_{v0}^2), in the rate of change before and after the change point CP_i (σ_{v1}^2 and σ_{v2}^2 respectively), and in the change point itself (σ_{CP}^2), respectively.

4.3.2 Model with group-effect

To explore heterogeneity in individual trajectories between groups of interests, the unconditional segmented growth model can be expanded by including time-varying covariates (TVCs) at level-1 and time invariant covariates (TICs) at level 2, while simultaneously adjusting for the effects of variables measured on participants at all time points. Whereas TICs directly predict the growth parameters, TVCs directly predict the repeated measures while controlling for the influence of the growth parameters [97, 132]. If the TIC variable is a binary dummy grouping factor ("GRP_i"), identifying participants coming from two identified groups, the model can be rewritten as follows:

Level-1 model: (4.4)

$$Response_{ij} = b_{0i} + b_{1i}age_{ij}.(1 - u_{CP_i}(age_{ij})) + b_{2i}(age_{ij} - CP_i).u_{CP_i}(age_{ij}) + b.TVC_{ij} + \varepsilon_{ij}$$

Level-2 model: (4.5)

$$b_{0i} = \beta_{00} + \beta_{0grp}GRP_i + v_{0i}$$

$$b_{1i} = \beta_{10} + \beta_{1grp}GRP_i + v_{1i}$$

$$b_{2i} = \beta_{20} + \beta_{2grp}GRP_i + v_{2i}$$

$$CP_i = CP + \beta_{CP}GRP_i + v_{CPi}$$

Where β_{00}, β_{10}, β_{20} and CP are the expected trajectory parameters for the reference group (at zero values for other potential covariates); β_{0grp}, β_{1grp}, β_{2grp} and β_{CP} are the expected intergroup variations in these parameters for participants in the second group (i.e. respectively, in the mean response, in the linear age effect, in the deviation from linear rate after the CP and in the CP timing); and v_{0i}, v_{1i}, v_{2i} and v_{CPi} are the level-2 residuals person *i* for intercept, slopes, and age at the change point after controlling for group differences. To test for a between-group difference in one trajectory parameter only, '*GRP'* can be included as a level-2 predictor for the parameter of interest, and model all other growth parameters as random effects only (as in 4.2). For each of the *p*+1 individual growth parameters, additional participant-specific covariates (TICs) can be included in a similar fashion to have multiple predictors at level 2 as follows: $b_{pi} = \beta_{p0} + \sum_{q=1}^{Qp} \beta_{pq}\, x_{qi} + u_{pi}$, with x_{qi}, the qth measured TIC; β_{pq} ,the effect of the TIC x_{qi} on the *(p+1)th* trajectory parameter; and u_{pi}, the *(p+1)th* random effect. The set of p+1 random effects for person *i* assumed to be multivariate normally distributed with covariance matrix of dimension $(p+1) * (p+1)$, although simpler variance-covariance structures of the random effects can be considered during model building (i.e. mutual independence of the random effects). It is advised to standardise TVCs in order to stabilise the variance, improve normality of errors and linearity of the mean [132]. The common assumption for the error structure is $\varepsilon_{ij} \sim iid\ N(0, \sigma_e^2)$ but it can be relaxed to include time specific variances or residual error correlation such as AR(1) errors.

The same approach can be used to expand the hierarchical piecewise trajectory model with grouping factors that have more than two levels. This is one of the possible approaches to test for a cohort-effect on the development of curvilinear responses over time when data arises from multi-cohort or accelerated longitudinal designs. If study participants belong to one of *k* possible birth cohorts, *k-1* binary dummy variables are created to identify observations coming from people born in the same calendar year, and as in 2.2, these new *k-1* grouping variables are introduced as level-2 predictors of the different trajectory parameters in the model. The binary dummy variables are introduced to sequentially shift the conditional means of each of the different trajectory parameters. The fixed effects will be the average trajectory parameters for the cohort chosen as the reference cohort in the study sample, and each $(\beta_{cohort})_{1..k-1}$ coefficient will thus be interpreted as the variation in growth parameters in the corresponding *k-1th* cohort compared to the reference cohort. The fixed effects will be the average trajectory parameters for the cohort chosen as the reference cohort in the study sample, and

each $(\beta_{cohort})_{1..k-1}$ coefficient will be interpreted as the variation in growth parameters in the corresponding *k-1*th cohort compared to the reference cohort.

4.3.3 Trajectory divergence mechanisms

The equation 4.4 above, allows for between-group difference in each of the four trajectory parameters of the piecewise model (i.e. intercept, slope before and after the CP, and CP itself). If the focus is to determine and model the divergence in the trajectories between group, then model 2.2 can be modified by forcing the intercept and slope before the CP to be invariant across groups by setting β_{0grp} and β_{1grp} to zero at level 2 in equation 4.4. As illustrated in Fig 4.1, three possible ways in which continuous outcomes trajectories can diverge over time between groups are illustrated: (1) Type 1, the two groups have different slope after the CP; (2) Type 2, the two groups have different change points; and (3) Type 3, the two groups have different CP and post-CP slopes. To test for group-difference at different stages of the outcome development, the approach consists of fitting these three possible conditional Bayesian hierarchical models to the data and comparing model fit to determine which mechanisms provide the best representation of the underlying development of the outcome between groups of participants.

4.3.4 Bayesian estimation of the hierarchical piecewise model

The main technical challenge in multilevel models with random change points is that the likelihood does not have a closed-form expression, and parameter estimation often requires approximate likelihood or sampling-based inference methods [197, 201]. A Bayesian approach was chosen to estimate and summarize the parameters of interest in the conditional multilevel piecewise model (equation 4.4). With many random change points and participant-level trajectory parameters, repeatedly sampling from the conditional posterior distribution for each parameter in the model helps avoid the computational challenges of the ML framework when estimating models with a large number of random effects. In our illustrative example, all models were fit in `RJAGS` and `R2JAGS` in `R`, two packages that implement the Gibbs sampler for Bayesian inference and perform similarly to `WINBUGS` with the advantage of the flexibility of the `R` interface for the data manipulation and model formulation. In combined Bayesian

notation, the trajectory model with a binary grouping status 'GRP' as the TIC covariate interacting with all four trajectory parameters can be written as follows:

$$Response_{ij} \sim Normal(mu_{ij}, \sigma^2) \qquad (4.6)$$

$$mu_{ij} = v_{0i} + \beta_{0grp} GRP_i + (v_{1i} + \beta_{1grp} GRP_i) age_{ij} + (v_{2i} + \beta_{2grp} GRP_i)(age_{ij} - (v_{CPi} + CP_{grp} GRP_i))$$

To ensure that the effect of 'group' on each trajectory parameter can be either positive or negative and that the prior information does not dominate the likelihood, uninformative priors were set for the fixed group effects β_{0grp}, β_{1grp}, β_{2grp}, CP_{grp} as $N \sim (0, 10^4)$. In vector notation, the random effects $v_i = \left(v_{oi}, v_{1i}, v_{2i}, v_{cpi}\right)^T$ are assumed to follow a multivariate normal distribution with mean β and unstructured 4 x4 variance-covariance matrix φ as in 1.3, where $\beta = (\beta_0, \beta_1, \beta_2, CP)^T$, the vector of population means. Traditionally in Bayesian analysis for random effects, InvWishart(Σ,k) is used as a conjugate prior to the unknown variance-covariance matrix of multivariate normal distributions, where Σ is a positive definite inverse scale matrix of degree of freedom k [124]. Inverse-Gamma (λ_1, λ_2) is often used as the conjugate prior to the variance of univariate normal distribution (i.e. for mutually independent random effects, and model error variance σ^2). Alternative prior distributions may be used for level 2 variances of independent random effects or for the variance components of multivariate normal distributions [66, 124, 304].

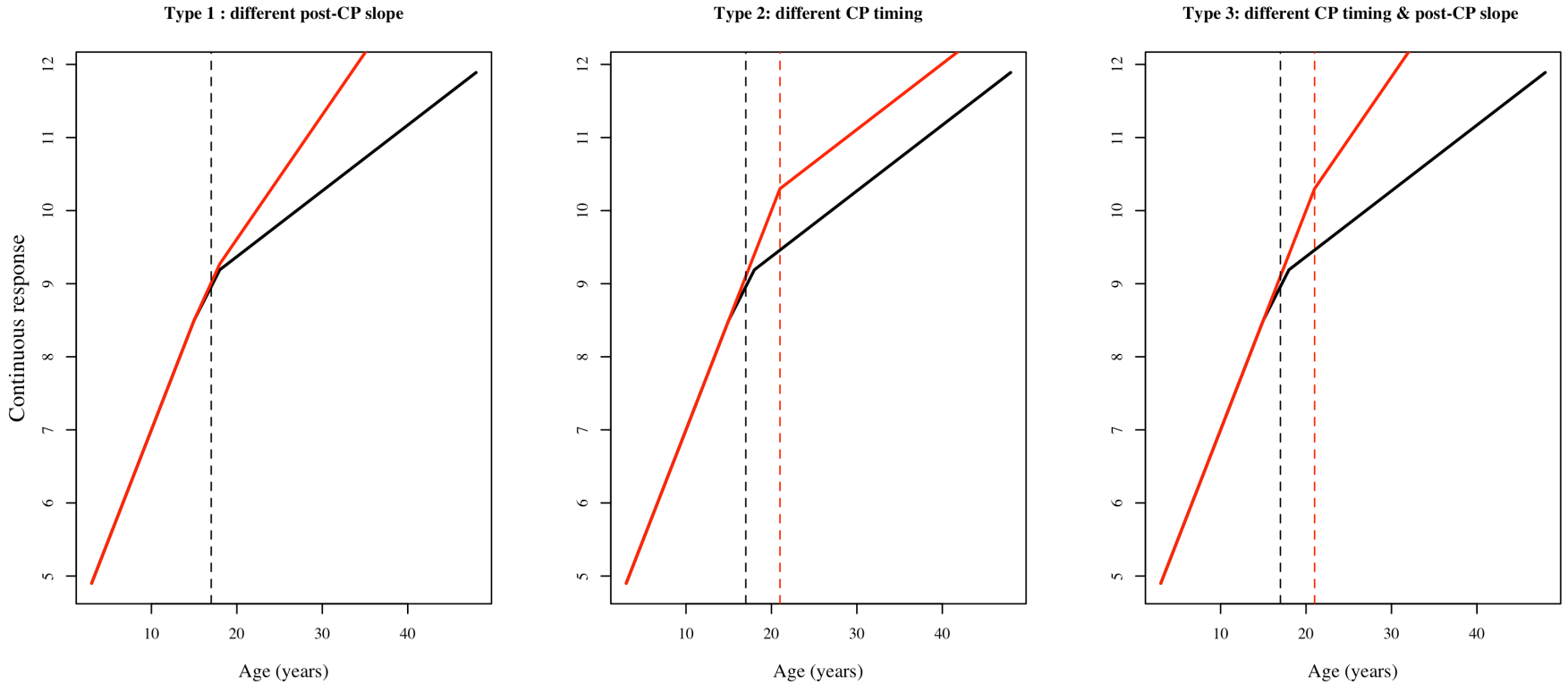

Fig 4.1. Three hypothetic models of between-group divergence in curvilinear response trajectories over time.

Red and black solid lines indicate the average response curve of participants belonging to one or the other group; dashed lines show the position and age at change point(s) for the two groups of participants, or the age at which trajectories diverge between the two groups. Graph obtained using simulated data.

4.3.5 Significance of group-differences in trajectory parameters

Testing for group-differences in trajectory parameters is equivalent to investigating the significance of the grouping covariates parameters at level-2 in the hierarchical change point model. In the Bayesian context, this is achieved by examining the posterior probability density for the "β_{grp}" parameters in 2.2. (i.e. β_{0grp}, β_{1grp}., β_{2grp}.and β_{CP}) of the estimated covariate parameters. For example, the effect of 'GRP' on each trajectory parameter is significant if the 95% Bayesian credible intervals (CI) of the estimated regressors (i.e. each " β_{grp}") exclude zero, in which case, the estimated " β_{grp}" can be interpreted as the shifts in each trajectory parameter in one group compared to the other group [124, 125, 305] .

4.3.6 Model convergence, fit and adequacy

The choice of the best model among the suite of candidate (conditional) Bayesian hierarchical models can be based on several criteria.

The first criteria is the deviance information criterion DIC [306, 307], a Bayesian equivalent of AIC, is an index of quality of fit that is commonly used for Bayesian model comparison [126]. DIC is a two-term measure of model fit or adequacy ($\overline{D}$ posterior expectation of the deviance) and a penalty term for increasing model complexity ($p_{D,}$ effective number of parameters), estimated as half the average within-chain variance of the deviances in JAGS (DIC = $\overline{D}$+pD). p_D can be interpreted as the expected reduction in uncertainty due to estimation. The interpretation of DIC is similar to that of the AIC as a summary of the relative fit between the model being tested and the 'true' model generating the data, while balancing model complexity with the information available from the data. The smaller the DIC, the better the fit. Appendix 4.7 shows a `R` function that was written to retrieve DIC directly from the MCMC samples in `RJAGS`.

A further way to investigate model adequacy in Bayesian analysis is to conduct posterior predictive checks of the likelihood of each fitted model. By looking for systematic discrepancies between real and simulated data, posterior predictive checks consist of simulating replicated data under each candidate model and comparing these to the observed data [66]. Although posterior predictive p-values (PP p-values) are not suitable for model comparison, they offer a reliable index of stand-alone model fit in that they estimate the probability that the data could have been generated under the candidate model. A PP p-value close to 0 or 1 suggests the observed data has an extreme test statistic and that the model may not be adequate. A PP p-value close to 0.5 means that the model predictions resemble the data. In this application, a posterior predictive plot and PP p-value were calculated in `jagsUI` for each candidate model, using the sum of residuals as a discrepancy measure.

4.3.7 Study sample

The application of the proposed Bayesian piecewise modeling approach is illustrated to investigate the divergence in child-to-adult trajectories of BMI between participants who do and do not develop adult T2DM in a well-studied ongoing population-based prospective cohort, the YFS [62]. Details on study design and on the collection of cardiovascular risk factors between 1980 and 2011 are published elsewhere [60] and summarised in Appendix 4.2.

In previously published research on the YFS cohort, Sabin et al. [292] showed that elevated BMI in children between 9 and 18 years was associated with an increased risk of developing T2DM in adulthood [292]. Additionally, a sex- and insulin-adjusted mixed model incorporating participants ages as a categorical variable suggested that differences in average BMI values between those who do and those who do not develop adult T2DM tended to emerge during adolescence, becoming marginally significant from the age of 15 years onwards. In this approach, the between-group

difference at each age group was assessed by pairwise comparisons of the predicted Least-square means, and did not incorporate BMI trajectory information at the individual or population level. In contrast, the proposed hierarchical piecewise regression approach considers and makes full use of individual trajectory information to test for group-differences at specific stages of BMI development from childhood to adulthood. Unlike categorical approaches, the proposed growth model provides a clearer representation of the underlying pathological BMI development among those who develop T2DM in adulthood.

In the illustration, data on 2540 YFS participants (1401 females and 1139 males) is included. These participants were followed-up a maximum of six times between 1980 and 2011 (Fig 1. in Appendix 4.1). Information on adult T2DM status was collected on participants at their latest individual adult follow-up (i.e. dichotomous outcome coded 0 for participants without T2DM and 1 for those with T2DM in 2001, 2007, or 2011). Included participants had at least one BMI measure available in childhood (i.e. in 1980, 1983 or 1986 between age 3 and 18 years). Participants had on average 4.98 repeated measures of BMI over the study period, with 90.7% of participants having 4 or more BMI measures (Fig 2. and 3. in Appendix 4.1 File). 88 included participants (3.5%, 44 females and 44 males) had T2DM in adulthood. BMI observations made among those aged 3 years in 1980 were excluded because only 3 participants in this birth cohort developed T2DM in adulthood. Furthermore, the lack of BMI measures between 3 and 6 years prevented modeling the downwards slope from infancy peak, nor the age at adiposity rebound, which usually occurs before age 6 years in normal weight children [14, 145]. Using BMI data collected on participants aged 6 years and over, it was assumed that most included participants had already reached this important childhood milestone, and that a linear trend was thus an appropriate functional form to model childhood BMI growth from that age. Fig 4.2. shows that individual BMI responses are not linear between 3 and 49 years of age and that there are important between-person differences in BMI trajectories.

Since sex differences in childhood growth and pubertal timing are well documented [308], subsequent BMI trajectory modeling between age 6 and 49 years was conducted among males and females separately [309]. BMI, especially in adulthood, is slightly right skewed, but using log10 transformed BMI in the modeling approach presented below did not alter the conclusions drawn. For ease of interpretation, the results are presented using untransformed BMI only.

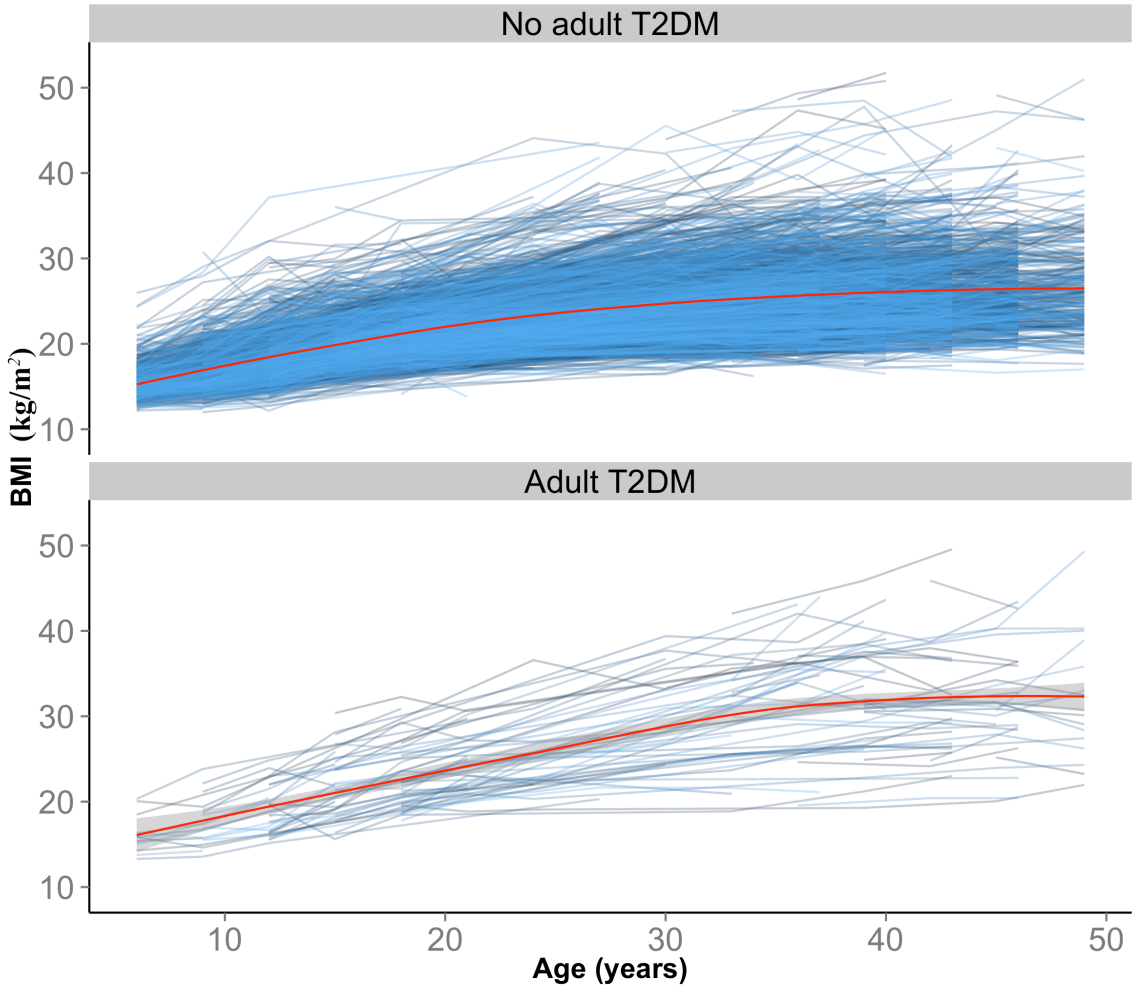

Fig 4.2. Spaghetti plot of the individual trajectories of those with T2DM in adulthood (N=88) and those who did not develop T2DM in adulthood (N=2452).

Red solid line: loess smoother curve indicating the average longitudinal trend in each group.

Visual inspection of the sex-specific smoothed BMI trajectories confirms the presence of a divergence between the two groups in adolescence (Fig 4.3). Compared with participants who remain healthy, those who develop T2DM seem to have greater average BMI levels by the time they are young adults, although it is unclear whether this divergence results from a group-difference in the timing at which the transition to a slower BMI growth rate happens (Type II divergence) from a group-difference in rate itself after puberty (Type I divergence), or from both (Type III divergence).

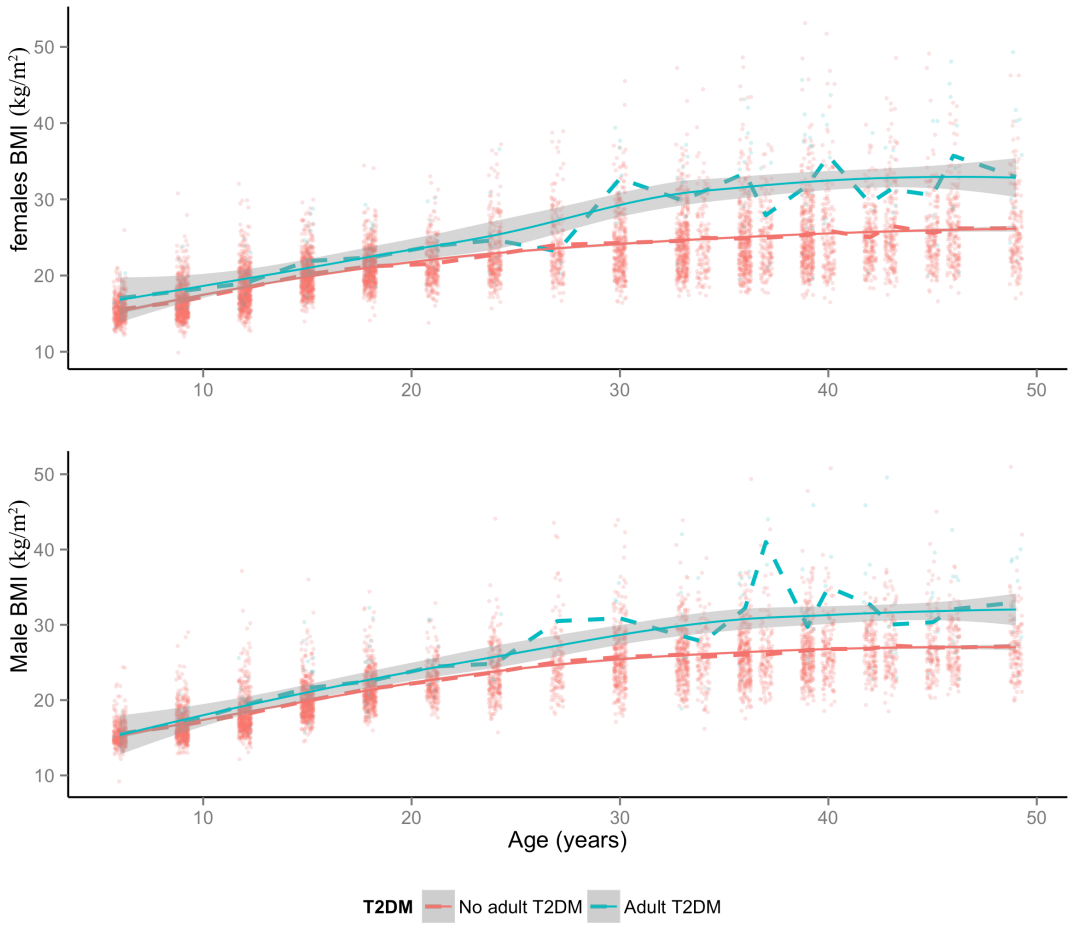

Fig 4.3. Scatterplot of the life course BMI data (in kg/m^2) stratified by sex.

Solid lines and grey bands: loess smoothed average trajectories and confidence intervals for each group (adult T2DM vs. non-T2DM group); dashed lines: age-specific averages of BMI levels.

Although the distal outcome of 'adult T2DM' is the grouping factor of interest in this illustrative trajectory divergence analyses, it is also demonstrated how the same modeling approach can be used to investigate potential inter-cohort variation in childhood to adulthood BMI trajectories by considering models with 'year of birth' as a categorical level-2 predictor of each of the four trajectory parameters. Individual age- and sex-specific BMI Z-scores at baseline (in 1980) were also included as level-2 predictors of each BMI trajectory parameters to investigate if systematic deviation from participants of comparable age and sex at baseline had any influence on the development of BMI trajectories later in life. All continuous covariates used in the analyses were standardised in order to stabilise the variance, improve normality of errors and linearity of the mean.

Specific values used for the hyperparameters used in the illustrative analyses are provided in Table 4.1, with normal distributions parameterised in terms of a mean and a precision (i.e. 1/variance), and multivariate normal distribution parameterised in terms of a mean vector and precision matrix. While in principle φ can be unstructured, when applied to the serial BMI YFS data, convergence for some parameters could not be reached when considering an unrestricted covariance structure between all four random effects in the unconditional change point model (equations 4.1 and 4.2), probably due to over parameterisation. Because initial analyses suggested a correlation between the slopes before the change point (b_{1i}) and the difference in slopes after the change point (b_{2i}), the model was constrained by including a non-zero correlation between these two random effects but setting independence for all other random effects, leading to a block diagonal structure $of\ \varphi$ (Table 4.1). Based on DIC, this covariance structure was preferred over mutually independent random effects for both males and females (Appendix 4.5), and used

when expending the trajectory models with level 2 predictors. In this application, prior sensitivity was investigated by fitting the unconditional BMI trajectory model using three sets of priors for the hyperparameters (Appendix 4.5). Because that the choice of hyperparameters had a minor influence on the marginal posterior distributions, for subsequent (conditional) analyses, reported posterior estimates of parameters were estimated using the set of priors that yielded the lowest DIC in the sensitivity analyses (Table 4.1). In this set, the priors for the means of the change points were based on the sex-specific estimates that maximize the profile log likelihood for the fixed (population-average) breakpoints in the unconditional model (estimated at 16 years for females and 22 years for males, see estimation method in Appendix 4.4). Using these priors for the change point means also kept computation running times reasonable.

Table 4.1 Priors and hyper priors used in the conditional trajectory models analyses. (Note that priors listed for β_{grp} refer to both $\beta_{T2DMgrp}$ and all β_{cohort} in the analyses).

Second stage: Priors for random effects

$$\begin{pmatrix} b_{1i} \\ b_{2i} \end{pmatrix} | \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} \sim MVN\left\{ \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix}, \Sigma_{\beta 1 \beta 2} \right\}$$

$$b_{0i} | \beta_0, \tau_{b0} \sim N(\beta_0, \tau_{b0})$$

$$CP_i | \mu_{CP}, \tau_{CP} \sim N(\mu_{CP}, \tau_{CP})$$

Third stage: Hyperpriors for population parameters

$$\begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} \sim MVN\left\{ \begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 10 & 0 \\ 0 & 10 \end{pmatrix} \right\}$$

$$\Sigma_{\beta 1 \beta 2} \sim \text{invWishart}\left\{ \begin{pmatrix} 0.1 & 0 \\ 0 & 0.1 \end{pmatrix}, 2 \right\}$$

$\beta_0 \sim N(25, 0.001)$ and $\tau_{b0} \sim$ Gamma $(0.01, 0.01)$

$\mu_{CP} \sim N(16, 0.001)$ (for females) or $\mu_{CP} \sim N(22, 0.001)$(for males) and

$\tau_{CP} \sim G\text{amma}(0.01, 0.01)$

All $\beta_{grp} \sim N(0,0.001)$ (i.e. $\beta_{0grp}, \beta_{1grp}, \beta_{2grp}, CP_{grp}$ for each growth parameter)

$\beta_{initialBMI-z-score} \sim N(0,0.001)$

And $\varepsilon_{ij}|\tau_i \sim N(0,\tau_i)$ & $\tau_i \sim$ Gamma (0.01,0.01)

Priors for time-varying variables included in the analysis (sex- and age-specific BMI z-scores at the first visit and birth cohort) were set to N~ (0,0.001) for all corresponding parameters (i.e. all β_{cohort} priors and $\beta_{initialBMI-z-score}$). To remain consistent with previous analyses of these data [292], time-varying measures of fasting insulin were log-transformed and standardised before being included as a level-1 predictor in the Bayesian hierarchical models to improve right skewedness and to linearize its relationship with BMI. About 17% of the insulin measures were not available in the data. The missing data mechanism for insulin was considered non-informative, as there was no reason to believe that the probability of an individual insulin measure being missing depended on the true value of this missing insulin observation (although it may be related to other observed variables for that individual). Insulin was therefore considered to be missing at random (MAR), and a prior was specified for this covariate [310]. Since *log (insulin)* is approximately normally distributed, we specify a $N\sim(\mu_{log(insulin)}, \tau_{log(insulin)})$ likelihood for $log(insulin)_i$ and place a vague prior on its variance (i.e. $\tau_{log(insulin)\sim Gamma(0.001,0,001)}$). Under this parametrisation, the posterior predictive distribution for $\mu_{\log(insulin)}$ and $\tau_{\log(insulin)}$ is informed by the observed part of the data only. Although individual insulin measurements change at each data collection point, by adding *log(insulin)* as a level-1 covariate in the multilevel model, the estimated relationship between insulin and BMI development remains constant across time [23]. This is a reasonable assumption in this application, since data exploration did not suggest any systematic patterns of change in insulin levels at the intra-individual level as people age. That is, the age smoother estimate obtained by fitting a generalised additive mixed model had an estimated degree of freedom (*edf*) close to 1 and was not

significant (*p-value* >0.3), which did not suggest a non-linear relationship between *log(insulin)* and age [277].

Approximate posterior distributions of the parameters of models considered throughout the analyses are obtained via MCMC simulations. Each model ran with four independent parallel chains of the Gibbs sampler (see Appendix 4.6 for an example of code). For each model, the first 50000 iterations were discarded in a burn-in run, and the draws from the posterior were thinned by a factor of 10 to reduce serial correlation of the chains. The following 20000 iterations were used to obtain posterior distributions of the model parameters and individual-specific random effects by mixing the four sequences. Visual checking of multi-chain parameter MCMC iterations traceplots and the Gelman-Rubin diagnostic $\hat{R}$ <1.1 were used to assess that the chains converged to stationary distribution [124]. Residual errors were plotted to confirm they approximately followed a normal distribution.

4.4 Results

4.4.1 Divergence of BMI profiles in T2DM and non-T2DM YFS participants

Following the modeling approach presented in the Methods and the priors and their corresponding hyperparameters (Table 4.1), the following set of conditional Bayesian hierarchical piecewise models was fitted for each sex: unconditional (Model A), adult T2DM status adjusted intercept (Model B), adult T2DM status adjusted childhood slope (Model C), adult T2DM status adjusted adult slope (Model D), adult T2DM status adjusted CP (Model E), adult T2DM status adjusted CP and adult slope (Model F), adult T2DM status adjusted change point, childhood and adult slopes (Model G), adult T2DM status adjusted intercept, and change point (Model G), and a model with all four parameters adjusted for adult T2DM status (Model H). As mentioned above, previous research on these data set suggested BMI levels were not significantly

different between the two groups in childhood [292]. Models C (i.e. group difference in childhood slopes) and B (i.e. BMI response consistently higher in one group across the life course) were thus fitted to demonstrate the modeling approach. An annotated extract showing the RJAGS code syntax used to fit Model E is available in Appendix 4.6.

For both sexes, the lowest DIC was obtained when fitting model E, which was also the best fitting model with PP p-values close to 0.5 (Table 4.2). Posterior density plots of all estimated parameters, traceplots of the four chains across the 20000 iterations are shown in Figure A and B in Appendix 4.8. Additional model diagnostic statistics such as the Geweke, Gelman-Rubin, Heidelberger-Welch, and the Raftery-Lewis diagnostics, respectively, are shown in Appendix 4.9, and a pairwise correlation plot of all model parameters is given in Appendix 4.10.

The best fitting model, Model E, supported the Type II divergence mechanism where a difference in BMI levels emerged between the two groups due to a group difference in the change point timing. BMI growth rate in adulthood for both sexes was decreased by two-thirds compared with childhood (i.e. 0.67 vs. 0.18, and 0.61 vs. 0.15 kg/m^2 per year in childhood and adulthood for females and male, respectively), and participants who developed T2DM had similar BMI yearly rates of increase in adulthood compared with those who remained healthy (β_{2T2DM} effect not significant in model F for both sex Table 4.3). However, females who developed T2DM reached their developmental transition in BMI rate on average 12.37 years later (Table 4.3).

Table 4.2. Analyses of the divergence in BMI trajectories between T2DM adults and non-T2DM adults: assessment of Bayesian model complexity (effective number of parameters pD), and fit (deviance information criteria DIC) for each candidate model. Reported for each model are DIC(pD), and posterior predictive p-values (PPp-val). Best fitting models are indicated in bold characters.

	Model	**Females** DIC(pD)	**PP p-val**	**Males** DIC(pD)	**PP p-val**
Unconditional	A	26910 (2544)	0.47	19837 (2223)	0.55
T2DMgroup (int β_0)	B	26670 (2366)	0.45	19741 (2270)	0.43
T2DMgroup (childhood slope β_1)	C	26780 (2510)	0.6	19865 (2247)	0.58
T2DMgroup (Adulthood slope β_2)	D	26701 (2401)	0.58	19828 (2242)	0.62
T2DMgroup (change point CP)	E	**26076 (2777)**	**0.52**	**19762 (2213)**	**0.51**
T2DMgroup (CP + β_2)	F	26504(2430)	0.6	19860 (2271)	0.54
T2DMgroup (CP + β_0)	G	26436 (2751)	0.55	19896 (2242)	0.45
T2DMgroup (all 4 parameters)	H	26532 (2978)	0.52	19920 (2435)	0.51

Similarly for males, estimated BMI growth rates were not markedly different between the two T2DM groups in childhood or in adulthood, and were comparable with those estimated in females (Table 4.3). Compared with healthy adults, those who developed T2DM reached their slower BMI growth rate on average 6.47 years later.

The effect of the time-varying covariate of insulin at level 1 was significant for both males and females, with a 1-sd increase in *log(Insulin)* resulting in a BMI observation increased by 2.6 and 2.8 kg/m^2 respectively (i.e. $exp(\beta_{log\ (insulin)})$, Table 4.3). To assess potential differences in the magnitude of the insulin effect as a function of between-person characteristics, model E was expanded by including an interaction between 'adult T2DM status' and *log(insulin)*. For each sex, the estimated parameters

were not statistically significant (95% CI included zero), suggesting that the effect of insulin on BMI was homogenous between the two groups and across sexs.

The estimates of the variance-covariance parameters of model E, showed that the correlation between an individual's BMI growth rate in childhood and adulthood is equal to 0.61 for females and 0.47 for males, suggesting that children who have greater yearly BMI increase rates also have greater adult rates of increase (correlation estimated as: $\sigma_{\beta 1 \beta 2} \Big/ \sqrt{\sigma_{\beta 1}^2 * \sigma_{\beta 2}^2}$, Table 4.3). The between-participant variation around the change point σ_{CP} was comparable between males and females (Table 4.3). Fig 4.4 shows the estimated population-average prototypical trajectories for each sex and T2DM group, obtained from the estimated parameters for Model E, along with 100 trajectories predicted for each sex and T2DM group from Model E by Monte Carlo simulation. This illustrates a range of credible individual profiles generated under this model.

Table 4.3. Posterior mean parameter estimates for Bayesian hierarchical Piecewise BMI trajectory for best fitting trajectory divergence models in males and females (Models E). Posterior standard deviations (uncertainty in the parameters) are reported in brackets (Posterior mean (PSD)). Reported β_0 coefficients are in kg/m^2, β_1 and β_2 are in kg/m^2 per year, CP and CP $_{T2DM}$ are in years. All σ coefficients are standard deviations for the corresponding growth parameters and the residual error. $\beta_{\log(\text{insulin})}$ coefficients are in kg/m^2 for a 1 sd increase in *log(insulin*) level.

		Females Model E mean (PSD)	Males Model E mean (PSD)
β_0	I	26.5 (0.20)	27.46 (0.16)
β_1	S1	0.67 (0.012)	0.61 (0.01)
β_2	S2	-0.49 (0.015)	-0.46 (0.06)
CP	CP	16.02 (0.29)	21.62 (0.42)
CP $_{T2DM}$	CP	12.37 (1.21)	6.47 (1.23)
$\sigma_{\beta 0}$		2.07 (0.05)	2.36 (0.07)
$\sigma_{\beta 1}$		0.02 (0.005)	0.06 (0.004)
$\sigma_{\beta 2}$		0.07 (0.006)	0.05 (0.004)
$\sigma_{\beta 1 \beta 2}$		0.11 (0.05)	0.14 (0.03)
σ_{CP}		3.1 (0.26)	4.3 (0.2)
σ		1.33 (0.02)	1.21 (0.01)
$\beta_{\log(\text{insulin})}$		1.01 (0.04)	0.98 (0.03)

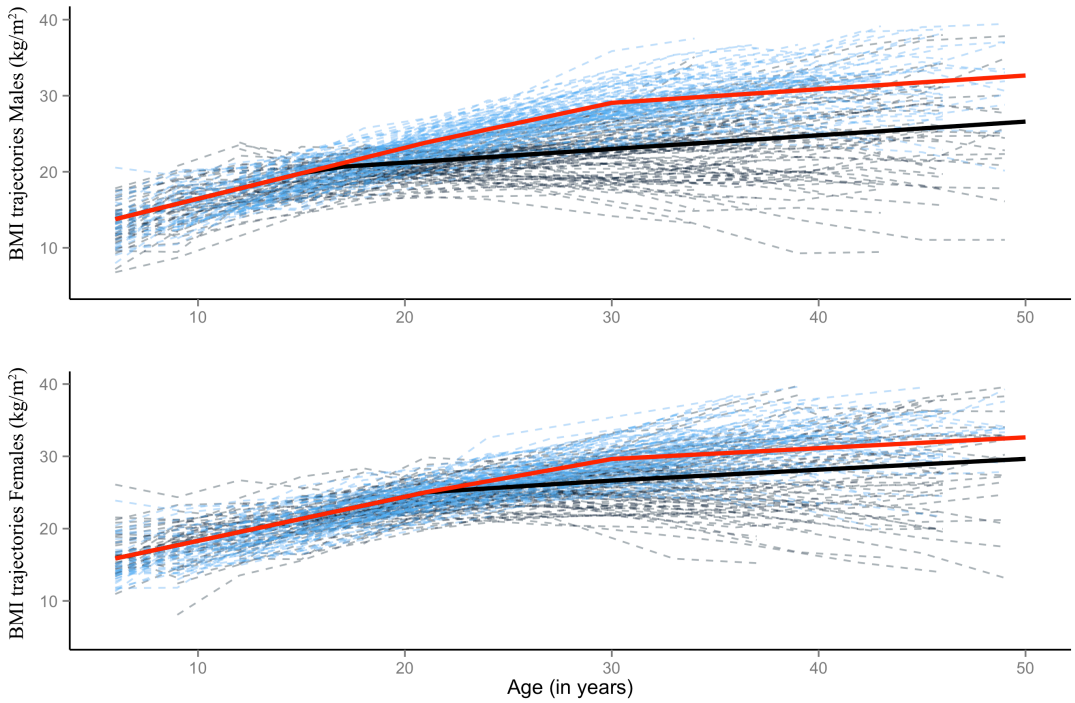

Fig 4.4. Sex-specific population average prototypical BMI trajectories for healthy and T2DM adults in the YFS cohort (solid blue and solid red lines, respectively) and prediction of 200 individual trajectories for each sex (100 per T2DM status group).

The dashed trajectories were obtained by MC simulation using sex-specific posterior estimates of mean and variance of growth parameters for the best fitting models (Model E). In these predictions, time varying measures of *log(insulin)* were set to the average *log(Insulin)* observed in the cohort.

For each sex and adult T2DM status group, Fig 4.5 shows a box and whiskers plot of the estimated individual BMI slopes obtained from Model E after the average change point in the healthy group and before the T2DM groups reach their average CP (i.e. slopes between 16.02 and 28.4 years in females, and slopes between 21.62 and 28.09 years in males). It illustrates that individual rates of change after puberty provides better discrimination of participants who went on to develop T2DM from those who

did not, compared with individual BMI levels at age 15 or 18 years for females, and ages 21 and 24 years for males (Fig 4. in Appendix 4.1). While the distribution of BMI levels at age groups surrounding the age at divergence overlap considerably, individual slopes allow to differentiate participants who have switched to a rate consistent with a normal slowing down of BMI development after puberty, from those who are still on the trajectory of increasing BMI.

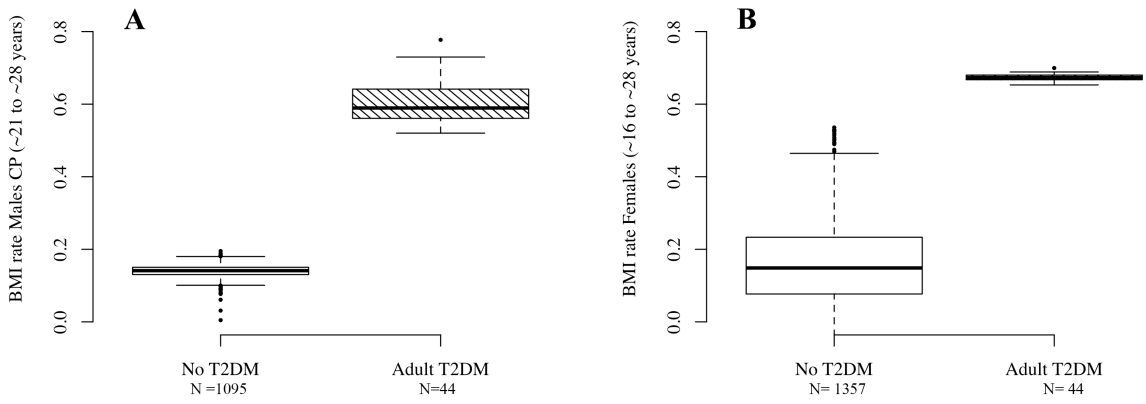

Fig 4.5. Box and whisker plots of fitted individuals random slopes between 16.02 and 28.4 years for females (A); and between 21.62 and 28.09 years for males (B).

Individual random slopes are estimated from the Bayesian hierarchical random change point model E. Solid lines in the boxplot indicate the group-specific median for the slopes (equivalent to the 50th percentiles of the posterior distribution).

4.4.2 Effect of age-and sex-specific childhood Z-score on BMI trajectories

To investigate if BMI z-scores in childhood predicted additional between-person heterogeneity in the trajectories of BMI, individual age-and sex-specific BMI z-scores at the first clinic were included as continuous level-2 predictors of each of the four growth parameters in sex-specific models. The only significant effect observed was for the childhood BMI slope, with a 1-sd increase in BMI z-score associated with

a 0.056 (sd=0.012) and a 0.038 (sd =0.009) increase in childhood (in kg/m^2 per year) for males and females respectively. This suggests that in the YFS sample, higher age- and sex-adjusted BMI at first visit in childhood were associated with faster BMI increase in childhood, but not with the age at transition in BMI development nor the change rate in adulthood.

4.4.3 Between cohort heterogeneity in BMI trajectories

Although the loess smoothed birth cohort-specific BMI profiles did not suggest obvious divergences in BMI trajectories between birth cohorts (Fig 4.6), five binary dummy variables identifying BMI observations of people born in different years (i.e. 1962, 1965, 1971, 1974 and 1977) were introduced as level-2 predictors of BMI growth parameters in sex-specific models (with year 1971 as the reference level) in a similar manner, to test whether year of birth was associated with between-participant heterogeneity in the development of BMI from age 6 to 49 years. The following set of Bayesian hierarchical piecewise models was fitted separately in males and females: unconditional (Model A); birth cohort adjusted intercept (Model B); birth cohort adjusted childhood slope (Model C); birth cohort adjusted adult slope (Model D); birth cohort adjusted "Age at transition in BMI growth" (i.e. change point CP) (Model E); birth cohort adjusted change point and adult slope (Model F); and birth cohort adjusted change point, childhood and adult slopes (Model G). Convergence was not reached for the most complex model where all four trajectory parameters (i.e.β_0, β_1, β_2, and CP) were adjusted for birth cohort effects.

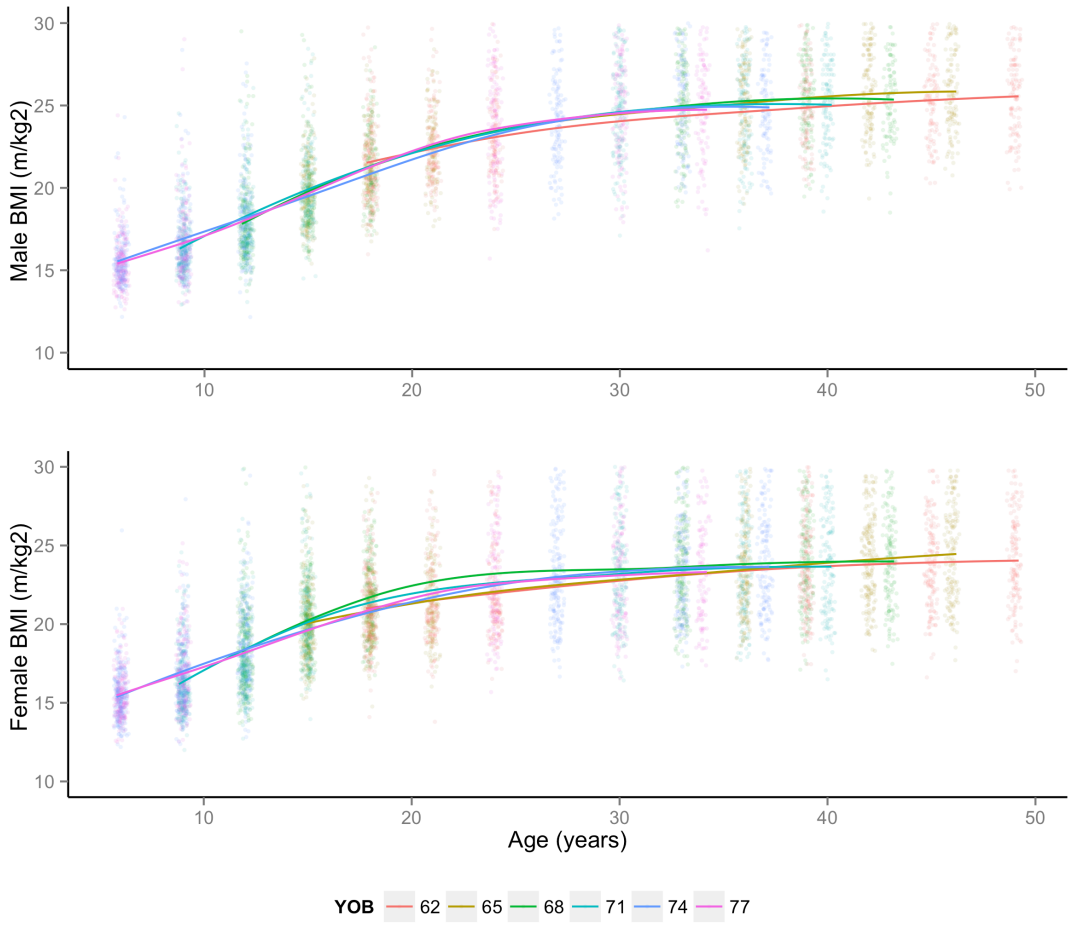

Fig 4.6. Birth cohort-specific scatterplot and loess smoothed BMI profiles (solid lines). YOB: year of birth (in years).

To improve readability, confidence intervals around cohort-specific smooth lines are not shown in this figure, and the upper range of displayed BMI data has been limited to 35 kg/m^2.

As can be seen in Table 4.4, the models with the lowest DIC varies between males and females. Increasing the complexity of the model did not improve model fit for males, and the lowest DIC is obtained for the unconditional model (Model A) suggesting that their life course BMI trajectory is more stable across birth cohorts. For females, model E marks a significant improvement in model fit, suggesting that the most significant predictor of between-cohort variations reside in the timing of the

CP, although the best model was obtained when adjusting for a cohort effect on both the adult BMI growth rate and CP.

Table 4.4. Analyses of inter-cohort differences in BMI trajectories: assessment of Bayesian model complexity (effective number of parameters pD), and fit (deviance information criteria DIC) for each candidate model.

Reported are: DIC(pD), and posterior predictive p-values (PP p-val) .Best fitting model for each sex indicated in bold characters.

	Model	**Females** DIC(pD),	**PP p-val**	**Males** DIC(pD),	**PP p-val**
Unconditional	A	26910 (2544)	0.72	**197837(2223)**	**0.52**
Birth cohort (int β_0)	B	26811 (2455)	0.70	19872 (2232)	0.70
Birth cohort (childhood slope β_1)	C	26759 (2489)	0.34	19849 (2175)	0.63
Birth cohort (Adulthood slope β_2)	D	26645 (2358)	0.67	19857 (2263)	0.68
Birth cohort (change point CP)	E	26395 (2599)	0.60	19862 (2211)	0.63
Birth cohort (CP and β_2)	F	**26390 (2671)**	**0.49**	19877 (2255)	0.43
Birth cohort (CP, β_2 and β_1)	G	26783 (2775)	0.48	19945 (2342)	0.53

For each sex, the posterior mean parameter estimates of the best fitting model are presented in Table 4.5. The results show that most of the between cohort variation for females is due to slight trajectory differences in two specific birth cohorts: those born in 1968, who reached the transition to adult BMI growth rate on average 2.89 years later than the 1971 birth cohort, and those born in 1974 who had adult BMI yearly rates increased by 0.06 (e.g. adult slopes of 0.24 compared with 0.18 kg/m^2 per year on average for the other 5 birth cohorts). Because inter-cohort differences in BMI development over age were not pronounced, cohort effect was not adjusted for the models estimating the effect of T2DM status on BMI.

Table 4.5. Posterior mean parameter estimates for best fitting birth cohort adjusted trajectory model for each sex; Model E for females and Model A for males.
Posterior standard deviations in brackets. $^{\xi}$ 95% credible interval includes zero (reference cohort is 1971). Reported β_0 coefficients are in kg/m^2. All β_1 and β_2 coefficients are in kg/m^2 per year, CP coefficients are in years. σ coefficients are standard deviations for the corresponding growth parameters and the residual error. $\beta_{\log\,(\text{insulin})}$ coefficients are in kg/m^2 for a 1-sd increase in log(insulin) level.

Parameters	**Females (Model E)** mean (PSD)	**Males (Model A)** mean (PSD)
β_0	26.92 (0.27)	26.510 (0.17)
β_1	0.63 (0.017)	0.62 (0.011)
β_2	-0.45 (0.02)	-0.48 (0.01)
CP	16.04 (0.5)	21.83 (0.46)
β_2 yob62	0.011 (0.01) $^{\xi}$	-
β_2 yob65	0.01 (0.01) $^{\xi}$	-
β_2 yob68	0.03 (0.025) $^{\xi}$	-
β_2 yob74	**0.06 (0.02)**	-
β_2 yob77	0.01 (0.03) $^{\xi}$	-
CP yob62	0.24 (0.69) $^{\xi}$	-
CP yob65	0.53 (0.4) $^{\xi}$	-
CP yob68	**2.89 (0.8)**	-
CP yob74	0.33 (0.8) $^{\xi}$	-
CP yob77	0.7 (0.73) $^{\xi}$	-
$\sigma_{\beta 0}$	2.08 (0.05)	2.38 (0.06)
$\sigma_{\beta 1}$	0.01 (0.003)	0.07 (0.004)
$\sigma_{\beta 2}$	0.18 (0.005)	0.05 (0.01)
σ_{CP}	3.22 (1.07)	5.79 (0.21)
$\sigma_{\beta 1 \beta 2}$	0.11 (0.05)	0.14 (0.03)
σ	1.37 (0.03)	1.21 (0.02)
$\beta_{\log\,(\text{insulin})}$	1.00 (0.04)	0.99 (0.03)

4.4.4 Simulations

A short series of simulations was conducted to compare difference in estimates of the age at which the groups diverge when using the proposed Bayesian piecewise growth modeling approach compared to a more traditional approach based on pairwise comparison of LS-means estimated from a categorical mixed model. Repeated measure data was simulated from a Type II divergence model (i.e. group-difference in the change point timing only), using the posterior estimates of mean growth parameters for the model fitted for females (average parameters are set to: β_O= 26.5, β_1 = 0.67, CP = 16.02, $\beta_{GroupCP}$=12.37, β_2 = -0.49, matching Model E posterior estimates for females in in Table 4.3), and both a participant-level random effect ($\sigma^2_{error} = 2.77$) and an observation-level residual error ($\sigma^2_{error} = 2.47$). Under this model, "CP*",* the change point for the first group to depart from the population-average childhood slope represents the age at which the two groups of participants diverge in their outcome trajectories (i.e. the second group maintain their increase his rate of change for 12.30 years longer). To closely resemble the YFS BMI data, ages at the first visit for each participant was simulated by randomly sampling baseline ages of real YFS participants. These simulated baseline ages were then subtracted by 25 years, and data was simulated as 6 non-missing repeated measures 3, 6, 9, 21, 27, and 31 years later for each participant. We considered 3 scenarios of sample sizes for the number of participants in each group (group 1/group 2): (1) 100/100, (2) 50 /100, and (3) 30/100. For each of the three scenarios, we simulated 100 datasets and fitted both a mixed model with age as a categorical variable and the Type II divergence Bayesian Hierarchical piecewise model using the set of priors defined in Table 4.1 For each piecewise model, we recorded the posterior estimate for the "CP" parameter, and for each fitted categorical mixed model, we applied pairwise comparison of the least-square means (LS-means) with Tukey adjustment for multiplicity to retrieve: (1) the earliest age at which the group-difference in means was found significant ($p<0.05$); and (2) the midway point between two consecutive ages that had a minimum number of non-significant differences in means before, and significant

differences in means after. The "midway point" method (2) is a potential alternative definition of age at which the group-difference appears in the LS means. Compared with the " earliest age with p-val<0.05" method (1), the "midway" point definition minimises the impact of simulations where some tests show significance at a young age, even though tests for the surrounding ages are not. For each scenario, estimated ages at divergence using the three methods were averaged across the 100 simulations. Fig 4.7. presents the simulation results in term of the quartiles distribution and means of these estimates of age at divergence across the 100 simulations.

When sample size decreases for one group of participants, the pairwise LS mean comparison method will tend to overestimate the age at divergence, with significant variability in the estimates arising due to random variation, especially when age at significance is determined using the first age at which a p-value <0.05 occurs (Fig 4.7). In contrast, the hierarchical Bayesian piecewise model was less sensitive to sample size, and the true age at divergence is consistently within the estimated interquartile range of the produced estimates, indicating that the Bayesian trajectory divergence model outperforms the LS mean method in both accuracy and precision, regardless of the way "age at divergence" is defined from the model output.

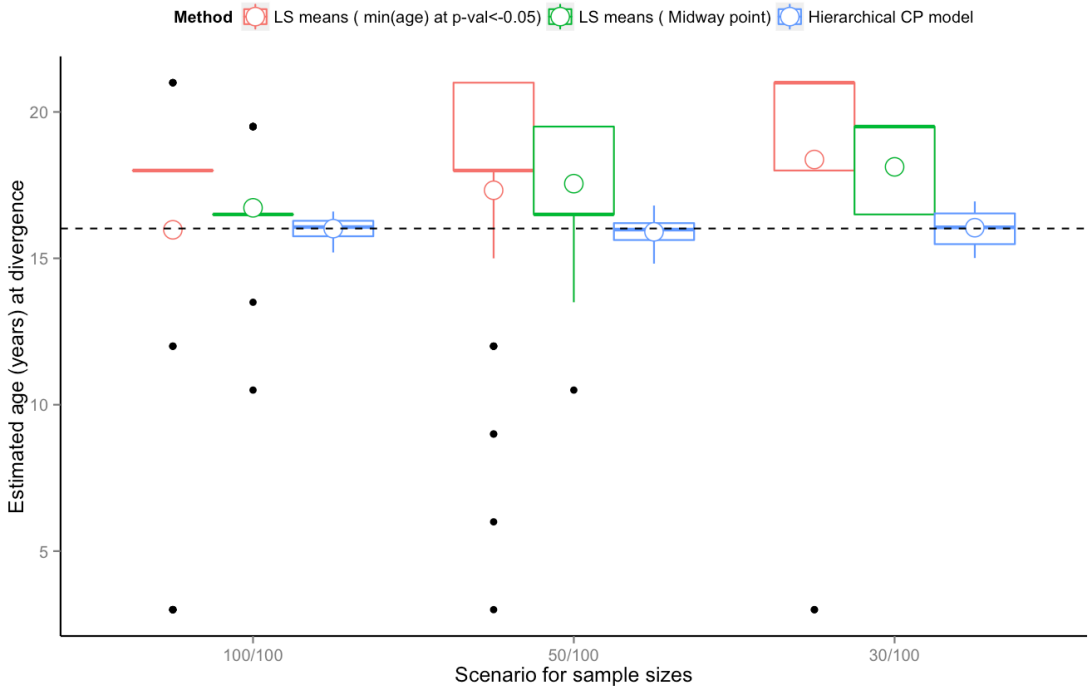

Fig 4.7. Boxplots and mean "Age at divergence" (x) estimated across 100 simulations using the 3 methods.

Bottom and top of the boxes are the lower (*Q1*) and upper quartiles (*Q3*), respectively; the bands near the middle of the boxes are the medians, the lengths of the boxes represent the interquartile range (*IQR =Q3-Q1*); the upper whiskers are defined as *min(max(x), Q3 + 1.5 * IQR)* and the lower whiskers as *max(min(x), Q1 – 1.5 * IQR)*. Means of age at divergence across the 100 simulations for each scenario are indicated with empty circles. The horizontal dashed line indicates the true age at divergence set in the simulations (16.02 years old).

4.5 Discussion

Using the repeated BMI data from the YFS study, we demonstrated how Bayesian hierarchical piecewise regression (BHPR) modeling may be used to investigate between-group trajectory divergence in non-linear longitudinal outcomes that are marked by developmental transitions.

The non-linearity in BMI development across the life course is well documented in the literature, with changes in BMI corresponding to a number of identified developmental phases [188, 308, 311]. In particular, BMI rate decelerates after puberty once people reach their adult height, translating to a leveling-off of the BMI trajectory in adulthood [163, 189]. Although many recent applications have relied on such approaches [15, 145, 146, 165, 312, 313], traditional polynomial parameterisations of growth curve models are not well suited to analyse BMI development, especially if the focus is to identify transitional changes or determine divergence between groups.

In contrast, piecewise linear regression is useful to break up a non-linear or curvilinear trajectory into separate linear components. For example, it allows comparison of the growth rate of different periods [191], marked either by a transitional change or separated by an experimental intervention point, creating a discontinuity in the outcome [182]. Piecewise regression is particularly suited to model BMI across different life-stages as its parameters map onto what is known about the natural development of BMI over time [25]. Since 'change points' (or milestones in the case of BMI) are model parameters in the piecewise model, there is no need to use elaborate techniques to retrieve these points of interest [102, 103, 165]. Piecewise models are also often preferable to more general continuous non-linear models if the number of repeated measurements per participant is small (i.e. 3 to 6 data points each as in [163, 187]) as is often the case in long-running observational prospective studies [145, 165].

Piecewise linear trajectory models, also known as linear spline, broken-stick and change point models [314], have been used to model developmental and longitudinal processes both in the MLM and SEM methodologies, with a wide range of applications in cognition [154, 190, 200, 201] and HIV research [196, 198, 199], and in prostate cancer detection [193]. Piecewise linear regression has been used to model sex-specific BMI developmental trajectories from childhood to adulthood in the multilevel setting [163, 187], where the breakpoints represent the transition in BMI growth at adolescence, but the position of the change points in these applications was based on likelihood

profiling [315] or fixed at given ages chosen based on previous knowledge derived from developmental theory [187]. In many instances, allowing individual heterogeneity in the change points, and modeling of the age at transition at the individual-level, is more likely to provide an adequate representation of non-linear processes over time, since for many continuous outcomes, participants may vary in the age at which their rate changes.

This chapter demonstrated how piecewise multi-level regression models may be used to characterise the divergence mechanisms in non-linear responses between groups by modeling change points as random parameters and introducing grouping factors as predictors of the between-person heterogeneity in responses over time. Although the main goal was to characterise how and when the developmental patterns of BMI diverged between those who did and did not develop T2DM in the YFS, the utility of the method to investigate cohort effects in the outcome response was also demonstrated.

Previous analyses of the YFS BMI and T2DM data considered categorical mixed models and tested for differences in the estimated BMI levels between the two T2DM groups at different ages by pairwise comparisons of the BMI predicted marginal means (i.e. Least-Square means) averaged over sex while adjusting for multiple testing (i.e. Tukey adjustment). This approach suggested that from age 15 years, the T2DM group had significantly higher BMI levels than those without T2DM. However, these analyses ignored the potential confounding effect of birth cohorts, and each existing "age" was treated as a non time-ordered categorical variable so that no inference could be made on individual or group-specific age-related BMI trajectories. Some age groups comprised those from up to five separate birth cohorts, while others only comprised those from a unique birth cohort (i.e. those aged 3 and 27 years). Having substantially fewer participants in one or both T2DM status groups at some age points results in a decreased power to detect a significant difference between groups (i.e. the observed difference at age 27 years was not significant in

either sex-averaged or sex-adjusted LS-means, Table 1 and Fig 1. in Appendix 4.3). Because BMI development is known to progress differently in males and females, and the oldest and youngest cohorts in the YFS sample are almost a generation apart (~15 years), not taking these confounders into account may result in biased inferences. In fact, when adjusting the LS-means for sex, the age at which the difference between T2DM groups becomes significant is not as clear since in males the difference is not significant at age 21 and 24 years, suggesting the true divergence in BMI between T2DM groups for males occurs more around those ages (Table 1 and Fig 2 in Appendix 4.3).

In contrast, the method illustrated here is not as sensitive to sample size and uses developmental theory to inform a model that allows between-group differences in within-person BMI trajectories at four possible levels for males and females to be examined (i.e. the overall BMI level, the childhood BMI growth rate, the adult BMI growth rate, and the age at which the transition between the two phases of change occurs).

Applied to the example data set, this novel approach allowed us to characterise group differences in the non-linear development of BMI and to identify a critical age window at which weight intervention programs might be best applied to help reduce or delay the incidence of T2DM in adulthood. The findings support the theory that girls who keep on gaining weight at the same rate they did in childhood past the age of 16 years are more likely to develop T2DM in adulthood. Similarly, for males, the natural deceleration in BMI velocity occurs, on average, at 21 years of age. Those who stay on their childhood BMI trajectory past that age may be at increased risk of developing T2DM.

Longitudinal studies often aim to make inferences on differences among average population health marker trajectories. Typically, this involves comparing change rates (or slope differences) in healthy participants vs. those with pathological development,

specific treatment conditions, or groups following certain lifestyle patterns. Using our Bayesian hierarchical piecewise regression approach, serial measures of patient's weight and height, often routinely collected in paediatric, general practice, and healthy or well child clinics, could be used to determine if an individual is on a path to an healthy adult weight status, or if their BMI trajectory places them in a category more susceptible to develop adult metabolic conditions such as T2DM.

4.6 Strengths and limitations

This study has developed a novel GCM approach to assess the mechanisms of trajectory divergence between groups of participants in long-running observational studies where the response profiles are non-linear. Simulation studies demonstrate that this novel approach allows overcoming the limitations of traditional methods often used to estimation of the age at divergence between groups of continuous response trajectories in the literature. This method is also flexible and can be extended to model response profiles with more complex non-linear shapes, and can consider more than two groups.

One potential limitation of this study is that the accuracy of the estimated age at divergence between the two groups may be hindered by the fact that BMI information was only available every 3 years. With higher resolution sampling (more available ages), the accuracy of the estimates would likely have been improved. Another limitation in this study is the lack of BMI measures in early childhood in the YFS (ages 3 and under). Under the premises of the foetal origin of adult disease hypothesis, it has been shown that birth size in conjunction with rapid compensatory growth in childhood can have an inverse relationship with health outcomes in later life [286]. It would be interesting to conduct further research in a cohort study with many individual BMI measures from birth throughout childhood to investigate

whether distinctive early-life growth patterns can be distinguished in those who develop adult T2DM.

4.7 Conclusions

Studying within-person and between-person differences in the development of continuous outcomes as a function of age in long-running multi-cohort observational studies is crucial to better understand the natural history of healthy vs. pathological risk factor profiles. Due to the typically unbalanced data designs, loss to follow-up and expected non-linear responses, it remains methodologically challenging to analyse such data. When the substantial focus is on when and how two or more groups of participants grouped according to a distal dichotomous health outcome have diverged in their response trajectories, traditional parameterisations of curvilinear growth model do not allow to identify an age at which the group that developed the condition moved onto a different path compared to the group that remained healthy. In contrast, the hierarchical piecewise multi-level modeling enables the separation of multiple aspects of change in complex developmental processes such as individual and group differences in the rates of change at different periods, and potential heterogeneity in the timing at which individuals from identified groups enter each developmental phase, providing a powerful tool to help inform intervention. Understanding fine-scale longitudinal change in risk factors trajectories over time may improve prediction of adult-outcomes, compared to approaches that summarize risk factor information by only looking at one childhood value, or a change in risk factor between specific ages. The methodology illustrated here focuses on a response with only one developmental change point, but it could easily be extended to more complex non-linear responses with multiple transitions.

KEY POINTS

- This chapter formulates a sequential Bayesian Hierarchical Piecewise Regression (BHPR) modeling approach to investigate and characterise the mechanisms of divergence in curvilinear longitudinal trajectories of continuous outcomes between two (or more) groups of participants.

- The proposed approach is applied to determine the age at which YFS participants who develop adult T2DM diverge in their BMI trajectories compared with those who do not develop T2DM.

- YFS participants whose BMI kept increasing at the same rates it did in childhood past age16 years for females and past age21 years for males may be at increased risk of developing adult T2DM.These data suggest weight interventions targeted at reducing the incidence of adiposity-related T2DM in adulthood should be undertaken before these ages

- In simulation studies, compared to multiple pairwise comparisons of predictions from categorical mixed models, the proposed BHPR modeling approach is less dependent on sample size and estimates the age at divergence between-groups more accurately.

- This chapter introduces and illustrates a novel person-based growth modeling approach based on linear-linear spline regression with random change points to investigate trajectory divergence between groups in long-term observational data, when the longitudinal continuous response is curvilinear.

Box 2. *Summary of key points from Chapter 4: Bayesian hierarchical piecewise regression models: A tool to detect trajectory divergence between groups in long-term observational studies*

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6. QUALITATIVELY DISTINCT CHILD TO ADULT BODY MASS INDEX (BMI) TRAJECTORIES AND ADULT CARDIOMETABOLIC OUTCOMES

6.1 Preface

This chapter builds on results from chapter 5 that suggested potential residual variability in the way BMI developed amongst the adiposity four groups of interest in the YFS defined using a two time point approach. To overcome this, a life course approach was used to characterise underlying between-person heterogeneity in long-term BMI patterns in the YFS cohort. Unlike the person-based Bayesian Hierarchical piecewise regression approach used in Chapter 5, Latent Class Growth Mixture Modeling (LCGMM), a group-based approach to trajectory modeling, allows participants who share similar features in their longitudinal response profiles to be grouped. Unlike the GCM approaches used in chapters 3, 4 and 5, LCGMM can be viewed as a semi-parametric clustering approach that allows the identification of sub-populations that are unable to be identified when using *a-priori* stratification of participants. To refine our understanding on how longitudinal BMI influences cardiometabolic profiles in mid-adulthood, latent BMI trajectory classes identified with this approach were introduced as predictors of important adult cardiometabolic risk phenotypes: dyslipidemia, high cIMT, hypertension and T2DM in logistic regression models.

In summary, in line with general aim 1 and 3 of this thesis, this chapter illustrates a relatively novel and complex person-based GCM approach, which allows the identification of clusters of distinct developmental trajectories that may exist in cohort study samples. In addition, it highlights how this approach can compliment other modeling strategies, resulting in enhanced understanding of the importance of developmental trajectories of cardiometabolic risk factors in the prediction of later cardiometabolic outcomes.

6.2. Introduction

Over the past three decades the prevalence of overweight and obesity have increased substantially in both adults and children, and these trends are predicted to keep increasing [40, 41, 52, 55, 60, 242, 249, 250, 290, 291, 332]. Excess weight and weight gain in children are associated with adverse cardiometabolic profiles in cross-sectional studies, and childhood obesity is known to track into adulthood [146, 316, 317, 319, 321, 323]. Childhood adiposity is associated with adverse long-term cardiovascular risk markers in adulthood and with increased CVD morbidity and mortality [40, 291, 316, 319, 320, 355-357].

To date, many epidemiologic studies examining associations between obesity and adult CVD risk have focused on assessing BMI at a single or limited number of time points [33, 43, 357, 358], ignoring the dynamic changes of BMI over time and the potential diversity in child to adult BMI developmental patterns.

Recent developments in statistical techniques allow the investigation of the heterogeneity of BMI trajectories that may exist within a population [223, 226, 231, 234]. A number of studies have prospectively explored BMI trajectories in the periods of early childhood crossing over adolescence using raw BMI, or BMI z-scores [8, 166, 234, 359], but none have examined a study period large enough to encompass the life course from young childhood until mid-adulthood. As a result, the progression of BMI from adolescence into adulthood is less well-described [16]. Recent data suggest that obese individuals that become non-obese between childhood and adulthood have a normalisation of adult CVD risk [326]. However, it is unknown if the shape of BMI developmental patterns across the life course, and in particular whether different patterns of high BMI incidence, resolution, or stabilisation from childhood to adulthood play a role in predicting cardiometabolic risk in middle-adulthood. From a public health perspective, determining whether developing or resolving abnormal BMI status at different ages is useful in predicting specific cardiometabolic risk in later life would help inform policy and interventions.

Qualitatively different child to adult BMI trajectory patterns are likely to exist within populations. We hypothesised that those with consistently higher BMI levels or faster rates of increase in BMI in early life would have greater CVD risk in mid-adulthood compared to either those who maintained an ideal BMI trajectory from childhood to mid-adulthood, and also those who develop overweight or obesity at an older age, and have experienced a shorter duration of adverse BMI status.

Using data from the 31-year prospective YFS study in which participants have up to eight measures of BMI from childhood onwards, the aims of this study were to (i) identify subgroups of participants who share similar trajectories in BMI from childhood though middle age and (ii) determine the independent association of these BMI trajectories with high cardiomeabolic risk phenotypes in mid-adulthood.

6.3 Methods

6.3.1 Study sample

This study considers a subset of 2631 YFS participants (1208 males and 1428 females) whose height and weight were measured on at least three occasions between 1980 and 2011 (1980, 1983, 1986, 1989, 1992, 2001, 2007 and 2011), including the initial childhood measure (at baseline in 1980) and at least one of the adult follow-ups (2001, 2007, or 2011).

BMI at each follow-up was calculated as $Weight\ (kg)/Height^2\ (m)$. At baseline in 1980, participants were aged 3 to 18 years, and at the most recent follow-up in 2011 were aged 34 to 49 years. On average, included participants had 5.4 individual BMI records and 72% had 5 or more BMI measures. BMI measures where excluded in 2001, 2007, and 2011 if participants were pregnant at the time of examination. Participants or their parents provided written informed consent, and the study was approved by local ethics committees (The Ethics Committee of the Hospital District of Southwest Finland) in agreement with the Declaration of Helsinki.

6.3.2 Definition of adult high cardiometabolic risk phenotypes

Adult cadiometabolic risk outcomes of diabetes, hypertension, dyslipidaemia, and increased cIMT (indicating pre-atherosclerosis of the carotid artery) were assessed in 2001, 2007 and 2011. For each participant, the presence of absence of each CVD risk at the latest available examination was determined using the following definitions [326]: Participants were classified as having T2DM if they had a fasting plasma glucose level of 7 mmol per litre (126 mg per decilitre) or higher, reported the use of oral glucose-lowering medication or insulin but had not reported having type 1 diabetes, or had received a diagnosis of T2DM from a physician at any of their adult follow-up examinations (2001, 2007 or 2011). Participants were classified as having hypertension if they had a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher, or if they reported the use of blood pressure–lowering medication. High-risk lipid levels (dyslipidemia) was defined according to NCEP guidelines, as LDL-C levels of 160 mg/dl (4.14 mmol/l) or higher, HDL-C levels of less than 40 mg/dl (1.03 mmol/l), and triglyceride levels of 200 mg/dl (2.26 mmol/l) or higher [245]. For LDL-C, participants who indicated they were currently taking lipid-lowering medication were classified as having high-risk levels. cIMT in adulthood was measured in the left common carotid far-wall using established protocols [12], with high cIMT defined as values equal to or greater than the 90th percentile for age-, and sex-specific values [326].

6.3.3 Statistical methods

Identification of latent BMI trajectories

Heterogeneity in the longitudinal development of BMI was investigated using LCGMM to identify subgroups of YFS participants who shared similar underlying BMI trajectories between 6 and 49 years of age. A series of LCGMMs considering several polynomial specifications of BMI as a function of age and a number of variance-covariance structures for the random-effects were fit using the `lcmm` package in `R` [360], which regroups a series of functions to estimate latent class mixed models for Gaussian and non-Gaussian longitudinal outcomes [361]. As recommended in the literature [220, 225, 226, 231, 362], various LGCMM models were fit and compared before choosing a final model. Participants' ages in the analyses were centrered around 25 years, the

mean age in the population sample, to facilitate the interpretation of the intercepts and improve numerical stability. Three possible polynomial specifications were considered to describe the within-person shape of the longitudinal BMI response as a function of age: a linear, and also a quadratic and a cubic specification, which allowed for curvilinear developmental patterns of BMI. Each of these polynomial models (order 1 to 3) were respectively modeled as a one to seven class solution, starting with a standard one-class heterogeneous mixed model where all subjects are assumed to follow the same underlying trajectory over time, and increasing the number of latent classes progressively.

For each of these models the specification of the intercept and slope(s) variance (i.e. random effects) was considered to allow for between-person heterogeneity in the BMI response. To limit the number of potential models tested, the variances of random intercept and slopes were constrained to be equal across classes, and the systematic approach of Feldman et al [363] was used to relax the constraints on the variance/covariance matrix. The linear, quadratic, and cubic growth mixture models were tested respectively with intercept variance only and with intercept and slopes variances. When relevant, each model was tested with all possible covariances estimated, and with all covariances constrained to zero. In general, the more estimated covariances; the fewer classes could be successfully extracted from the models, that is models with large number of classes failing to converged (i.e. singular matrix) or were unable to generate standard error of parameters. However, all classes could successfully be estimated when the covariances of all random effects were constrained to zero for all models (data not shown). All models presented in Appendix 6.2 were defined with a block diagonal structure for the random intercept and slopes, which represent varying but uncorrelated growth parameters.

As recommended by Proust-Lima et al. [361], models with two or more classes were ran several times with different sets of initial values to avoid convergence towards local maxima, a problem commonly encountered when fitting mixture models. Each model was fit with (1) initial values generated from the maximum likelihood estimates of the corresponding 1–class model, (2) initial values randomly generated from the asymptotic distribution of the corresponding 1-class model, and (3) initial values obtained via grid searching (with a maximum of 20 iterations from 30

random vectors of values from the 1-class model)[361]. The results of the series of models that were fitted solely to ensure the correct convergence of the program algorithm towards global maximum of the log-likelihood are not shown. The models results presented in chapter 6 are those of growth mixture models that showed satisfactory evidence of convergence.

For each model involving latent classes, posterior class-membership probabilities were used to obtain a posterior classification of the participants in each latent class to evaluate goodness-of-fit and to characterise the discrimination of latent clusters (See `lcmm` documentation for details on post-fit computations). The proportion of subjects classified in each class with a posterior probability above a threshold of 0.7 was also retrieved and indicates the proportion of subjects unambiguously classified in each latent class. Thirdly, the mean of posterior probabilities of belonging to the latent class among subjects classified a posteriori in each class was recorded (i.e. mean probabilities between 0.8 and 1 indicate a good classification). Model fit was further assessed by examination of model residuals and plots of weighted mean predictions of each model. As recommended in the literature, the choice of the best fitting model was based on BIC values, and on these other index of goodness of fit and discrimination [361]. When comparing different LCGMM models, it is recommended that to be considered a significant improvement over an existing model, a reduction of BIC of at least 10 points is necessary in the competing model [229].The best fitting model in terms of BIC were the 7 class quadratic and cubic models, but these models had poor discrimination for some classes (mean posterior probabilities <0.65 for several classes, less than 60 % of participants classified in several classes with a posterior probability above 0.7). Furthermore, when seven classes were considered, some classes contained less than 1% of participants. In contrast, the six class quadratic model had only a slightly larger BIC, a higher mean posterior probability in all six classes (> 0.78), and a high proportion of participants were classified in each of the six classes above the 0.7 threshold (70% of participants or above in each class). The parameter estimates of the fixed and random components of the six class quadratic mixture model are given in Appendix 6.3. The necessity of random intercept and slope variance within-class was assessed for this final model, but the results of these further analyses are not shown since these additional models did not result in a sufficient increase in BIC to justify class-specific variance components.

Additional details on the strategies used for model building, including choice of specification of functional form and variance-covariance structure of the model, identification of the optimal number of distinct latent classes, and the computation and analyses of post-fit indices is given in the Appendix 6.1.

Association with adult cardiometabolic outcomes

To determine the association between trajectory group and the different CVD risk outcomes in adulthood, the trajectory group memberships identified by LCGMM were introduced as predictors of each adult cardiometabolic risk in Poisson regression models with robust error variance [364]. In addition, for a subset of 2421 participants (N=1073 males), who had been assessed for all 6 high CVD risk phenotypes in mid-adulthood, a combined cardiometabolic load risk-score ranging from 0 to 6 was calculated as the arithmetic sum of the number of adverse cardiometabolic outcomes recorded for each participant at his/her latest adult follow-up (Appendix 6.6). The association between the BMI trajectory groups with the combined CVD-risk load variable (collapsed to 0, 1, and 2 or more risk phenotypes) was assessed using ordinal logistic regression. The adjusted models included year of birth (YOB) and sex as covariates. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit chi-square statistics.

6.4 Results

6.4.1 Latent Class BMI trajectories

Using BIC criterion, class membership posterior probabilities, and classification were used to assess the goodness-of-fit of the competing LCGMM models (Appendix 6.1 and Appendix 6.2), six discrete life course BMI trajectories were identified among the 2631 YFS participants considered in the analyses (Fig 6.1 and Fig 6.2): 55.2% of participants maintained normal weight status throughout follow-up (‘normal stable’ group (class 1, N=1453)), 33.4% followed a trajectory of increasing BMI that lead to overweight from the mid 30s (‘progressively overweight stabilising’ group (class 3, N=879)), 4.2 % had BMI levels increasing rapidly from

childhood, resulting in an overweight status in early adulthood and worsening obesity by early-mid adulthood ('progressively obese increasing' group (class 4, N=110)), 4.3% were borderline overweight in early childhood, overweight in mid-childhood and obese but stabilising by age 20 years ('rapidly overweight stabilising obese' group (class 5, N=113)), 1.2% followed a trajectory of persistent and increasing obesity throughout their life course, leading to BMI levels ≥ 40 kg/m^2 in mid-adulthood ('overweight / obese persisting' group, (class 6, N=33)), and 1.6% were overweight or obese in childhood increasing to obese by 25 years but progressively reversed their elevated BMI status between 30 and 50 years of age ('high BMI resolving' group (class 2, N=43)). Although some of these identified classes have low percentages of participants (<6%), they are highly discriminated with high mean a posteriori probabilities and high posterior probabilities (Appendices 6.1 and 6.2).

The parameter estimates of the fixed and random components of the six-class quadratic mixture model are given in Appendix 6.3. Basic characterisation of the participants in the identified classes showed that although the age range (6 to 49 years) was adequately represented in all the latent classes, there were differences in the average age across follow-ups, as well as the mean age at baseline among the classes. Sex differences were noted in specific classes of trajectories. For example, females were over-represented in the normal stable trajectory group (class 1), but the groups that stabilised their BMI in adulthood (i.e. 'progressively overweight stabilising' and 'rapidly overweight stabilising obese' groups (classes 4 and 5, respectively)) contained more males (Table 6.1). The incident "progressively obese increasing" group and the "overweight/obese persisting" groups also had more female participants, although females were also more likely than males to belong to the group who reversed their BMI status in mid-adulthood (high BMI resolving group, class 2, Table 6.1).

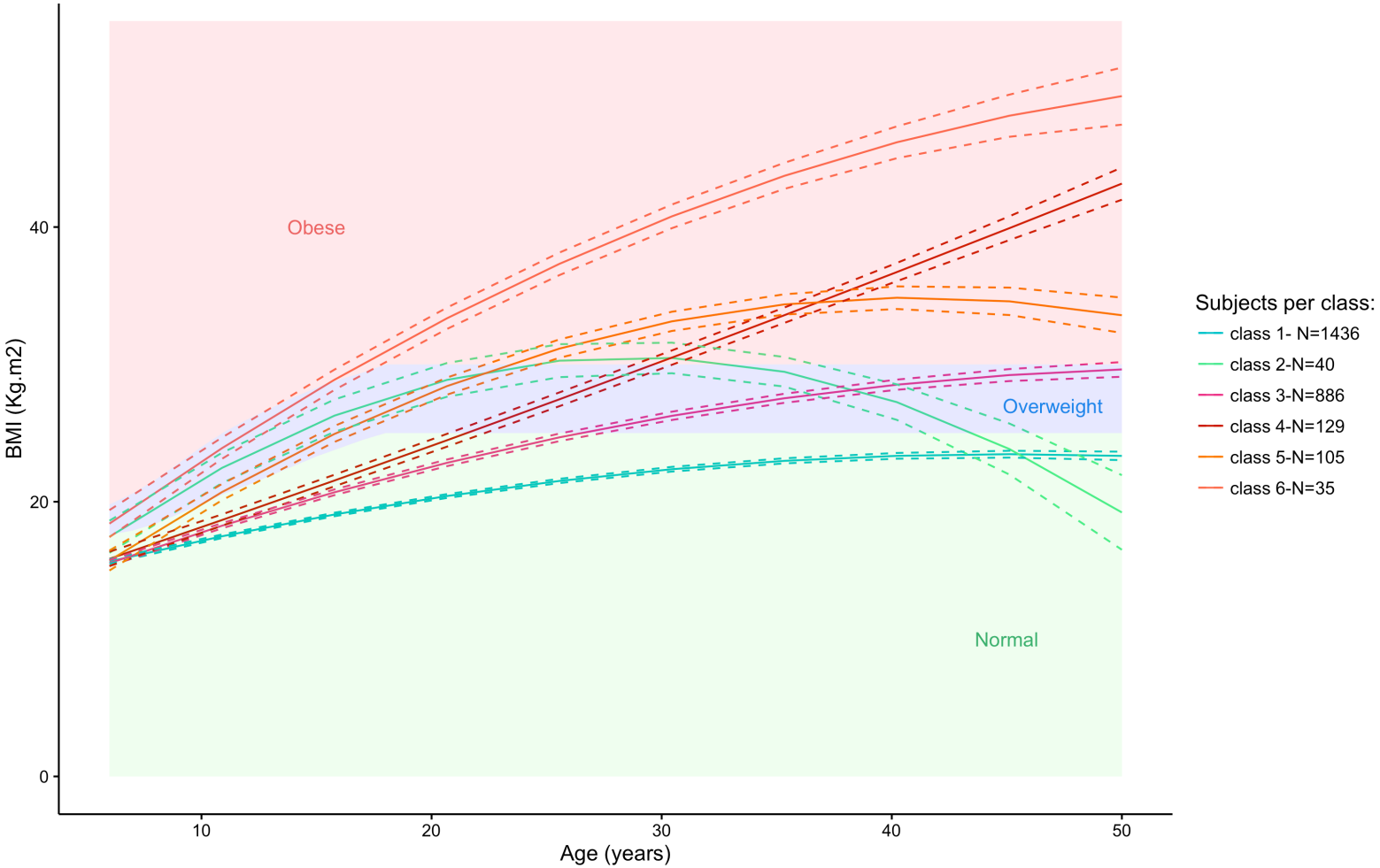


Fig 6.1. Class-specific mean predicted trajectories as a function of age in the best fitting six-class body mass index (BMI) growth mixture model (LCGMM) (solid lines) in the Cardiovascular Risk in Young Finns Study. Dashed lines indicate estimated 95% confidence intervals, and shaded background areas indicate normal, overweight, and obese BMI status across the life course (International childhood sex-specific cut points [338], were averaged across sexs at each age to improve readability). Number of participants attributed to each class is shown.

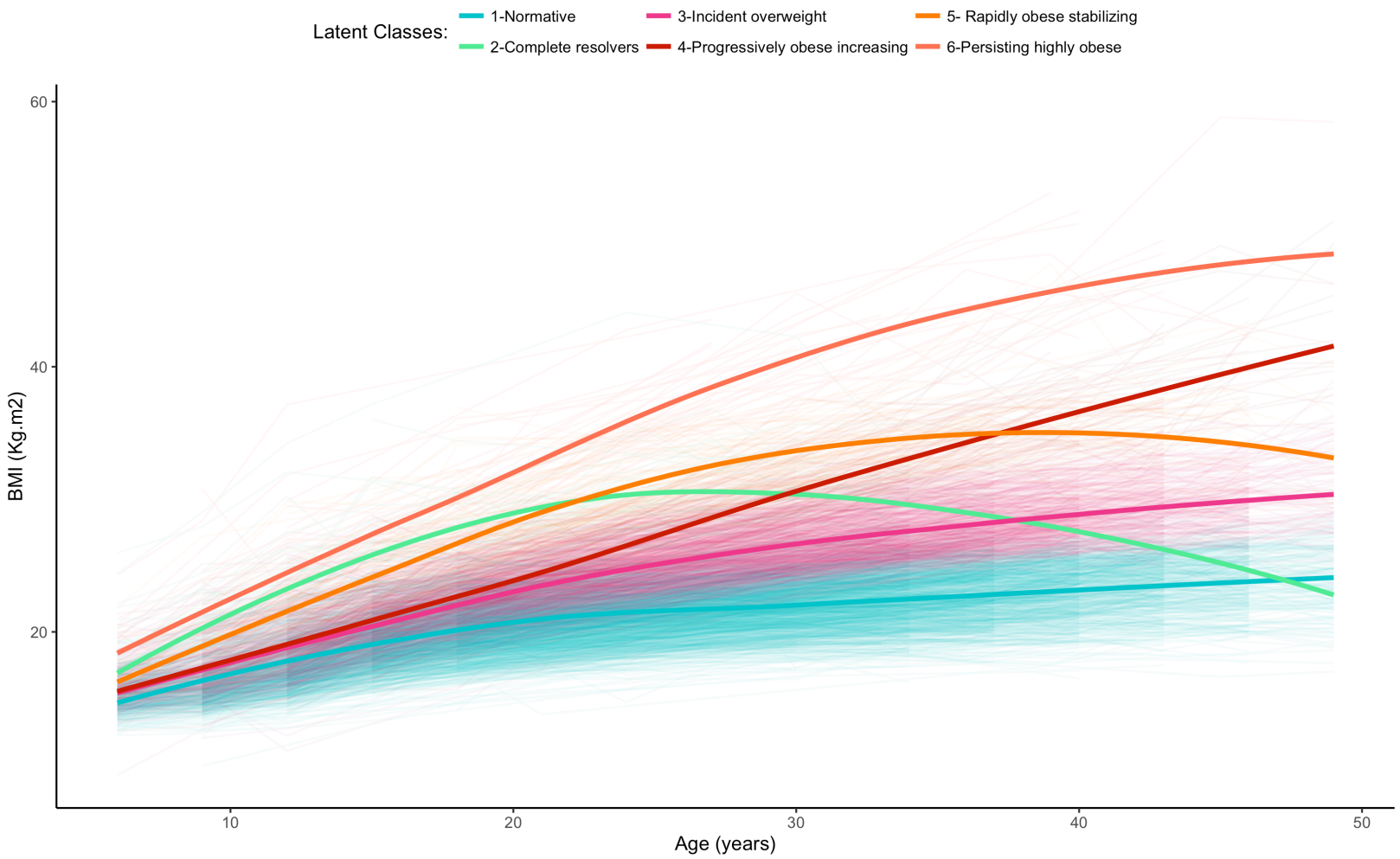


Fig 6.2. Latent trajectory classes identified for body mass index (BMI) between 6 and 49 years of age in the Cardiovascular Risk in Young Finns Study through latent class growth mixture modeling (LCGMM). Shown are the individual BMI profiles colour-coded according to posterior class membership (thin lines), and the loess-smoothed BMI trajectories for the six identified latent subgroups obtained by smoothing across all BMI data attributed for each class (solid lines).

Table 6.1. Basic demographic characteristics of YFS participants classified in the six identified latent BMI trajectory groups.

	Normal stable	Complete High BMI resolving	Progressively overweight stabilising	Progressively obese increasing	Rapidly overweight stabilising obese	Overweight / obese persisting	P-value*
	(class 1) N=1453	(class 2) N=43	(class 3) N=879	(class 4) N=110	(class 5) N=113	(class 6) N=33	
Mean age (sd)	25.1 (12.8)	22.3 (12.6)	21.4 (12.0)	23.6 (12.7)	24.7(12.5)	24.7 (12.5)	0.001
$Min_{(age)}$ –$Max_{(age)}$ (years)	6-49	6-49	6-49	6-49	6-49	6-49	1
Mean baseline age (sd) (years)	12.1 (4.2)	11.8 (4.0)	9.9 (3.8)	11.4 (4.0)	11.9 (3.9)	9.9 (3.6)	<0.001
Male, %	37.7	46.5	59.6	39	53.1	36.3	0.02

* Reported p-values from anova F-tests (for comparisons of means) and of chi-square tests of independence (for the comparison of proportions).

6.4.2 Association of BMI trajectory groups with high cardiometabolic risk phenotypes

Appendix 6.5 shows the prevalence of adult T2DM, hypertension, high-risk LDL-C, HDL-C, triglycerides level, and high-risk cIMT (marker of pre-atherosclerosis) in the subset of YFS of participants considered in the analyses. The prevalence of all 6 adult cardiovascular outcomes increased substantially between the group of participants who maintained ideal BMI levels across the life course (class 1) and the other BMI trajectory classes, with the highest prevalence obtained for the 'persistently overweight/obese' group (class 6), (Table 6.2). Compared with participants who were classified in the stable normal BMI trajectory class (class 1), all other classes had significantly higher risks for all considered adult outcomes, except for participants in the high BMI resolving group (class 2, N=43), for whom the difference in risk for T2DM, hypertension, and abnormal lipid levels was not statistically significant (Table 6.2).

Participants who completely resolved BMI in mid-adulthood (class 2, Table 6.2), had lower risks for developing adult T2DM than those who became overweight (class 3). Among those obese in adulthood, participants whose BMI kept increasing in adulthood (classes 4 and 6) had greater risks of developing T2DM compared with those whose obesity developed sooner in life but stabilised in their early adulthood (mid 30s) (class 5, Table 6.2).

For hypertension, the risk ratios were also smaller in those who normalised their BMI by mid adulthood (class 2) compared with the incident overweight participants (class 3), but they were incremental in classes 4, , and 6, suggesting the number of years spent obese may be an important determinant of adult hypertension (Table 6.2).

Table 6.2. Adjusted risk ratios (RR), 95% confidence intervals (CI) and Wald z-statistic p-values of the associations of BMI trajectory groups with high-risk cardiometbolic outcomes in adulthood and percent (%) of participants with the high risk outcomes in each BMI trajectory group.

Outcome and Latent BMI Trajectory group	%	RR[c]	95% CI[c]	P-value
Type 2 diabetes				
Class 1[a]	1.4	1[b]	-	-
Class 2	2.6	2.13	0.14 – 8.23	0.31
Class 3	3.5	2.49	1.38 – 4.58	**0.002**
Class 4	17.1	13.05	6.71 – 25.17	**$6.75.\ 10^{-15}$**
Class 5	12.6	9.33	4.39 – 19.08	**$1.1.10^{-9}$**
Class 6	20.1	19.45	8.63 – 31.16	**$7.5.10^{-10}$**
Sex Female	0.51	0.87	0.54 – 1.39	0.58
YOB	-	0.88	0.83 – 0.92	**$7.9.10^{-7}$**
Hypertension				
Class 1[a]	19.3	1	-	
Class 2	17.1	0.76	0.23 – 1.80	0.25
Class 3	26.9	1.64	1.36 –1.99	**$2.3.10^{-9}$**
Class 4	33.1	2.20	1.52–3.08	**$1.1.10^{-6}$**
Class 5	36.5	2.35	1.65–3.26	**$2.9\ 10^{-8}$**
Class 6	40.6	3.18	1.77–5.35	**$1.6\ .10^{-5}$**
Sex Female	41.3	0.54	0.44–0.66	**$3.3\ 10^{-9}$**
YOB	-	0.89	0.87 –0.91	**$2.1\ 10^{-16}$**
High-risk cIMT				
Class 1[a]	7.8	1[b]	-	
Class 2	25.1	3.37	1.80 –6.39	**$4.3\ 10^{-6}$**
Class 3	13.3	1.70	1.30–2.22	**$4.1\ 10^{-3}$**
Class 4	22.3	2.68	1.78 –4.40	**$1.3\ 10^{-6}$**
Class 5	24.5	3.27	2.11 –4.90	**$6.6\ 10^{-9}$**
Class 6	25.8	3.49	2.32 –5.71	**0.002**
Sex Female	0.52	0.97	0.75–1.25	0.8
YOB		0.98	0.95–1.01	0.2

Outcome and Latent BMI Trajectory group	%	RR[c]	95% CI[d]	P-value (*)
High-risk LDL-C				
Class 1[a]	9.4	1[b]	-	-
Class 2	10.5	1.03	0.14 – 1.10	0.10
Class 3	16.5	1.47	1.16 – 1.84	**$3.5.\ 10^{-5}$**
Class 4	17.9	1.59	1.37 – 1.95	**0.04**
Class 5	18.7	1.65	1.21 – 2.63	**0.006**
Class 6	19.8	1.78	1.11 – 2.72	**0.023**
Sex Female	34.3	0.41	0.32 – 0.52	**$1.4.10^{-12}$**
YOB	-	0.93	0.91 – 0.96	**$9.3.10^{-8}$**
High-risk HDL-C				
Class 1[a]	11.4	1	-	
Class 2	14.6	1.07	0.72 – 1.19	0.16
Class 3	26.3	1.57	1.16 –1.84	**$2.1.10^{-11*}$**
Class 4	41.8	1.75	1.04–12.1	**$1.1.10^{-16}$**
Class 5	39.9	1.72	1.10 –2.72	**$1.9\ 10^{-9}$**
Class 6	40.6	1.77	1.56–2.96	**$2.45\ .10^{-3}$**
Sex Female	51.5	0.26	0.21–0.32	**$2.3\ 10^{-12}$**
YOB	-	1.01	0.98 –1.02	0.49
High-risk triglycerides				
Class 1[a]	4.8	1[b]	-	
Class 2	4.5	0.48	0.09 –2.4	0.42
Class 3	17.7	3.06	2.31–4.10	**$2.1\ 10^{-15}$**
Class 4	27.7	5.62	3.61 –8.53	**$3.6\ 10^{-16}$**
Class 5	25.6	4.73	3.02 –7.23	**$4.3.10^{-15}$**
Class 6	18.9	4.03	1.56 –8.56	**0.0001**
Sex Female	28.4	0.34	0.25–0.45	**$4.1\ 10^{-12}$**
YOB		0.96	0.94–0.99	**0.01**

[a]Latent BMI trajectory classes are as follows Class 1, Normal stable trajectory (N=1359); Class 2, high BMI resolving (N=43); Class 3, progressively overweight stabilising (N= 879); Class 4, progressively obese increasing (N= 110); Class 5, rapidly overweight stabilising obese (N= 113); and Class 6, overweight/obese persisting (N=33).

[b]: The normative BMI trajectory class (class 1) is the reference group. Unadjusted models with only the trajectory groups were also fit but estimated Relative Risk Ratios (RRs) were not significantly different and the AIC suggested that the sex-and year of birth (YOB) adjusted models fit the data better (data not shown).

[c]The regression coefficients for each latent class, can be interpreted as the changes in relative ratios for belonging to a given class, vs. the reference latent class (class 1). For year of birth (YOB), the coefficients give the change in relative ratios of the outcome for a one unit increase in the predictor variable.

[d]The 95% CI for the relative risks was obtained by log-likelihood profiling of the robust standard errors.

Resolution of an elevated childhood BMI by mid-adulthood was not associated with a normalisation of the risk for cIMT, with participants in the high BMI resolving group (class 2) having nearly 3.5 times the risk for abnormal cIMT compared with participants in the normal stable BMI trajectory group (Table 6.2). Only those classified in the persistently obese increasing group (class 6) were at greater risk of developing greater cIMT in adulthood.

Compared with participants who followed a normative BMI trajectory throughout their lives (class 1), the risk of raised adult LDL-C levels was not significantly elevated among participants in the high BMI resolving group (class 2, Table 6.2), but they were approximately 1.5 higher risk in the incident overweight group (class 3). Those who were obese in adulthood were almost twice the risk of developing abnormal LDL-C levels, regardless of their BMI latent class (i.e. classes 4, 5 and 6). Participants who became overweight or obese had greater risks of having lower HDL-C levels in mid-adulthood, especially those whose obesity persisted from childhood to adulthood (class 6, Table 6.2). A high circulating triglyceride level was approximately 3 times more likely in the incident overweight group (class 3) compared with the normal stable group (class 1), but those with the highest risk ratio were the participants who became obese in adulthood (class 4, Table 6.2).

For the cumulative cardiometabolic risk load, the predicted probability plot derived from the proportional odd ratios estimated for the sex- and adult-age-adjusted ordinal logistic model (Fig 6.3) shows that the probability of observing a null cumulative cardiometabolic risk load (i.e. no cardiometabolic high-risk phenotypes in adulthood) decreases from 0.7 to 0.2 as participants BMI trajectory changes from normative ('normal stable' group, class 1) to the most adverse class ('overweight/obese persisting' group, class 6). That is, compared to those who maintain ideal BMI levels from childhood to adulthood, following a trajectory with consistently high or increasing BMI levels decreases the probability of having an ideal cardiometabolic profile in adulthood. Although the highest probability of having a single high-risk CVD phenotype in mid-adulthood was observed for participants who progressively became overweight (class 3), the probability of having 2 or more high-risk phenotypes in adulthood was 11 times higher in the 'overweight/obese persisting' group (class 6) compared with the 'normal stable' group (class 1).

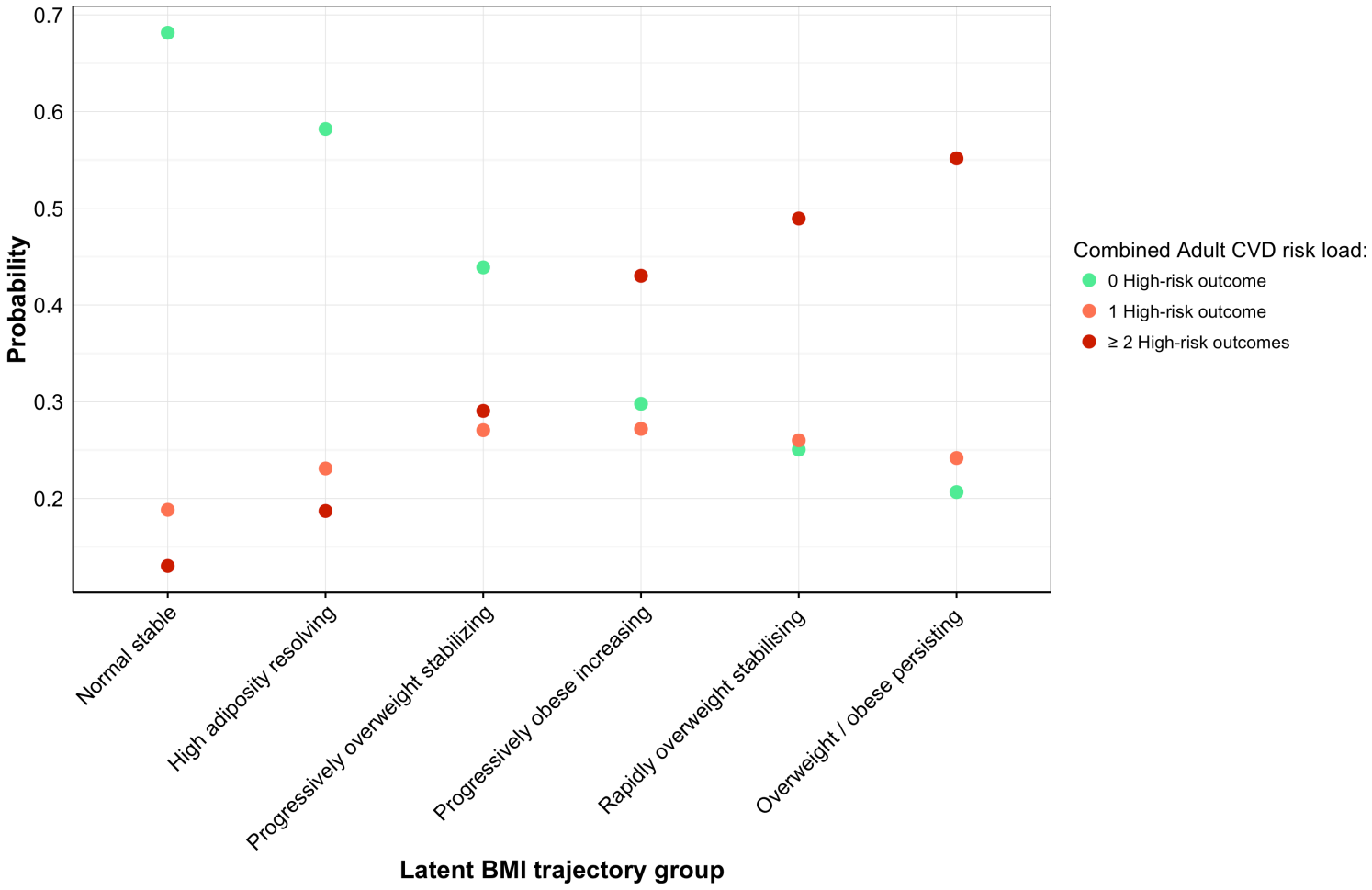


Fig 6.3. Predicted probability of having a cumulative cardiometabolic risk load of 0 (N=1360), 1 (N=540), and 2 (N=521) or more in the Cardiovascular Risk in Young Finns Study for each latent body mass index (BMI) trajectory class. Data derived from proportional odd ratios of a sex- and adult age-adjusted ordinal logistic model.

6.5 Discussion

In this chapter, LCGMM was used to identify six distinct underlying BMI developmental patterns in the YFS from age 6 to 49 years old, and determined the association between these identified patterns and adult risk of T2DM, hypertension, dyslipidemia, and high cIMT. The identified BMI trajectories were not readily identifiable in previous studies that used the subjective clustering of YFS participants into 4 a priori groups based on pre-determined BMI cutpoints at one point in childhood and one point in adulthood. Compared with previous approaches, this analysis allowed discriminating between truly normative BMI profiles (class 1) and BMI trajectories that lead to progressive but stabilising overweight status in adulthood (class 3). In addition, two groups with different patterns of obesity incidence were identified. One group of YFS participants had early onset of obesity but stabilisation to BMI levels to ~35 kg/m^2 in middle adulthood (class 5), and a second group that had later obesity onset, with adverse weight status appearing in their 20s, and BMI increasing throughout adulthood to reach worse asymptotic levels by mid-adulthood (class 4).

Higher trajectories of BMI were generally associated with increased cardiometabolic risk in middle age, which is consistent with findings of several studies that have shown excess BMI-years to increase risk of diabetes [16, 326, 365] and CVD risk.[326]. Participants who were normal weight in childhood but progressively became overweight in young adulthood (i.e. class 3), a group of individuals that could not be identified in previous analyses of this cohort in chapter 5 [33], had an increased risk in adulthood for all six high-risk cardiometabolic phenotypes considered. The prevalence of deleterious adult cardiovascular outcomes of developing T2DM, dyslipidemia and hypertension were higher among participants classified as progressively overweight (class 3) compared with participants who were obese in early life but who became normal weight by middle-age (class 2). These data suggest that completely reversing high BMI, even after childhood may lead to better outcomes than remaining stable overweight. However, incident overweight participants had less risk for high cIMT compared with the high BMI resolving group (class 2), suggesting that deleterious BMI status in early life may alter arterial structure in a way that is not reversible, even when normalising weight status later in life [52]. This finding is in keeping with a number of recent clinical studies suggesting that childhood obesity

may initiate pathogenic processes in the arterial wall that are persistent even in the face of improvements in body weight in later life [48, 50, 358, 366, 367]. Alternatively, childhood obesity may accelerate the progression of atherosclerosis in youth, leading to irreversible, established atherosclerotic lesions that can no longer be reversed by weight normalisation [47]. The findings reported in this chapter are consistent with an observational study that reported that elevated BMI in adolescence was associated with the risk of later coronary heart disease (CHD) independent of adult BMI levels. The authors concluded that the processes causing incident CHD, particularly atherosclerosis, developed gradually such that a longer history of relative overweight /obesity starting earlier in life contributed additional risk [16].

Of all cardiovascular outcomes considered, the highest relative risks were obtained for T2DM for two latent classes, the progressively obese increasing group (class 4) and the persisting overweight/obese increasing group (class 6) (RRs of 13 and 19, respectively, against other RRs < 3), suggesting that the absence of BMI stabilisation in adulthood, rather than the age of obesity onset, is very strongly associated with adult T2DM risk. This is consistent with an observational study reporting that the risk of diabetes was mainly associated with increased BMI levels close to the time of diagnosis [16]. In contrast, the results of this study suggest that the cumulative burden of the number of life-years spent obese may be a stronger predictor of the adult risk of hypertension.

The findings also suggest that the mechanism by which excess BMI may increase circulating LDL-C and triglyceride levels is primarily immediate and that a longer exposure to obesity does not additionally increase the risk of developing abnormal lipids beyond the level of BMI attained. Finally, analyses of the combined cardiometabolic outcomes indicate that completely resolving high BMI in later life may benefit cumulative cardiometabolic risk load in adulthood, with high BMI resolving participants (class 2) more likely to have no residual adult risk compared with incident overweight participants (class 3) who in comparison, had over 50% chance of having one or more cardiometabolic outcomes.

The current analysis provides additional clinically important granularity to the previous analyses of the YFS that suggested that overcoming excess adiposity in the time between childhood and adulthood leads to a normalisation of all cardiovascular risk phenotypes. However, since the study participants were subjectively categorised into four groups based on their movement in BMI status between two examinations (performed up to 27 years apart in childhood and adulthood), it was not previously possible to investigate the potential between-participants differences in BMI profiles that may have occurred in the interval between those two measurement time-points [326]. The way the 'high-adiposity resolving' group was defined in these previous analyses, did not allow to distinguish between 'true adiposity resolvers' (i.e. overweight or obese children who became normal weight adults), 'adiposity improvers' (i.e. overweight or obese children who became normal weight or overweight adults, respectively), or 'overweight persistent' (i.e. those overweight children who became overweight adults but did not develop obesity). Participants classified in a given BMI group, may also have resolved or developed elevated BMI at very different periods in their lives, in which case the age-BMI curves estimated for each group of interest may in fact be an amalgam of qualitatively different BMI pathways [223, 362]. In addition, despite a high prevalence of adult overweight in the different population samples (up to 36% overweight adults in the YFS, Appendix 6.4), the previous approaches collapsed adult overweight and normal weight into a single weight status category, preventing the discrimination of cardiometabolic risk between incident overweight and truly normative BMI trajectories. It is therefore possible that the definition used for the 'high-adiposity resolving' group and the potential misclassification of participants in this group, prevented the detection of the residual effect of elevated childhood BMI on adult cIMT risk in the previous analyses of this data [33].

Unlike traditional person-centered growth modeling approaches (including the Bayesian Hierarchical Piecewise regression approach used to model BMI trajectories in the YFS cohort in Chapter 5), group-based trajectory modeling techniques such as LCGMM can be used disentangle underlying population heterogeneity and move beyond a 'one size fits all' approach to describe risk factors trajectory development across the life course [231, 232, 368]. In classic approaches, the fundamental assumption is that all participants' trajectories can be represented

by a unique curve, so that any potential trajectory variations within-group will thus be absorbed by the model's random effects, representing the unexplained inter-individual variability around the average group trend [225] (see Appendix 6.1 for references and details). However, ignoring the potentially different ways in which obesity or overweight developed or normalised over time within an individual, and treating these as residual variance, may lead to a simplified or even biased picture of who is at increased risk in adulthood and the clinical relevance may be diluted. In LCGMM in contrast, the assumption is made that instead of the existence of a single developmental curve in the study population, individuals belong to distinct subpopulations with different developmental trajectories, and that individual children may have different pathways leading to the development of overweight/obesity [8, 20, 221, 223, 234].

Qualitatively distinct life course progressions of CVD risk factors have been shown to be associated with different levels of cardiometabolic risk later in life [13, 369, 370]. Consistent with the present findings, recent studies suggest that the cardiovascular consequences of obesity are possibly cumulative, and that the duration of obesity or overweight, notably, may be a stronger predictor of cardiometabolic outcomes in adulthood compared with a cruder measure of obesity-resolution or obesity-onset between two time points [356, 371-373]. Beyond the number of years spent living with an adverse weight status, the developmental period (i.e. childhood, puberty, mid-adulthood) at obesity onset, or the age at obesity resolution, may itself contribute to the strength of the association between the change in BMI status and adult outcomes, or reduction of risk [285, 325, 374]. To overcome issues associated with discrete categorisation of participants based on dichotomous measures of obesity or overweight at different ages [69, 354], a life course perspective may thus be useful to evaluate and refine the understanding of the impact of long-term BMI trajectory patterns on later-life cardiometabolic risk. Indeed, identifying groups of individuals with similar patterns of BMI over age in distinct BMI trajectories may compliment the classic individual-based approaches, as it may identify pathways of overweight onset and development during childhood and increase understanding of how weight status fluctuations impacts on cardiometabolic risk in adulthood.

In this chapter, the statistical approach allowed investigating of how distinct BMI trajectories across the life course influence later cardiometabolic risk. Although the observational nature of the data precludes any firm establishment of causality, it is hypothesised that stabilising BMI among obese adults could help limit their adverse cardiometabolic risk profiles. The reduction in all risk phenotypes except high-risk cIMT also suggests that the complete resolution of high BMI even in young adulthood is beneficial, although to effectively attenuate pre-atherosclerosis risk, obesity prevention should ideally target young children.

6.6 Strength and limitations

In recent years, a number of studies have explored BMI trajectories from infancy to early childhood, or childhood crossing in to adolescence [8, 166, 234, 238, 359, 368], but none covered a study period large enough to examine the heterogeneity in BMI trajectories from young childhood until mid-adulthood. In contrast, the 31 years of follow-up in the YFS cohort allowed us to investigate developmental trajectories of BMI between 6 and 49 years, providing an opportunity to detect and quantify the adult cardiometabolic risk in groups of participants that have not been well studied before: those who resolve their elevated childhood BMI in adulthood and participants who progressively became overweight.

This study also presents some limitations, in particular the lack of BMI observations in early childhood (< 3 years). As a consequence, this study did not allow the investigation of development of BMI across the entire life course, particularly during the critical period of adiposity rebound. In addition, these findings from a large cohort of European ancestry may not be generalisable to other study samples.

Further research should be undertaken to better characterise the YFS participants assigned to the six latent classes identified in this study, in particular, whether these BMI trajectory groups are distinguishable in terms of pre-existing participant characteristics (i.e. family history, parents BMI, socio-economic status), or their relationship to trajectories for other outcomes (i.e. latent life course trajectories of LDL-C, or diastolic blood pressure). More specifically, since our

results suggest at least two distinct BMI-age trajectories among incident obese subjects, if predictors of latent class membership can be identified, it may be possible to refine intervention strategies by targeting specifically children at higher probability of becoming obese at earlier or later points in life. This may improve the effectiveness of interventions on overweight prevention which have largely been of limited success, particularly in the long term [231].

6.7 Conclusion

BMI trajectories from childhood to adulthood vary, with trajectories that reach or persist at high levels associated with increased cardiometabolic risk in middle age. Findings suggested that the absence of BMI stabilisation in adulthood may be a stronger determinant of adult T2DM risk compared with the age at which obesity first developed. In additional, the risk for adult hypertension appeared to be stronger in those trajectory groups that developed high BMI early in life and have thus been obese for many years. The results suggest that stabilising BMI in obese adults could help limit adverse cardiometabolic risk profiles. The reduction observed for all outcomes except high-risk cIMT suggest the complete resolution of high BMI even in young adulthood may be beneficial to long-term cardiometabolic risk. However, the markedly increased risk for high-risk cIMT in middle adulthood despite body weight normalisation between childhood and adulthood emphasizes the importance of childhood obesity prevention to effectively attenuate the risk of pre-atherosclerosis, as well as the potential importance of identifying other modifiable childhood determinants of atherosclerosis development.

KEY POINTS – CHAPTER 6

- This chapter uses LCGMM, a group-based approach to GCM, to investigate between-person heterogeneity in the development of BMI trajectories in the YFS across the life course, and determined the association between different BMI patterns and adult risk of T2DM, hypertension, dyslipidemia, and high cIMT.

- Compared to the approach used in Chapter 5, where study participants belonged to one of four groups based on their change in BMI status between two time points, this approach considers all existing individual BMI observations to determine common patterns within the population, minimising loss of information and the issues related to subjective categorisation of participants in groups

- Six distinct BMI trajectories from age 6 to 49 years were identified in the YFS, including a trajectory of progressive overweight, which could not be discriminated from the truly normative BMI trajectory in Chapter 5, and two distinct trajectories leading to adult obesity.

- Higher trajectories of BMI were associated with increased cardiometabolic risk phenotypes in middle age. Completely reversing high childhood BMI may lead to better outcomes, however, the effect of deleterious childhood BMI status on high cIMT may not be reversible even with weight normalisation in later life. The risk for adult T2DM is strongly associated with the absence of BMI stabilisation in adulthood, rather than with the age of obesity onset.

- Completely resolving high BMI in later life reduced the cumulative cardiometabolic risk load in adulthood. Obese children who have normal adult BMI levels have less chance of having one or more cardiometabolic phenotype than participants who become overweight adults.

- This chapter illustrates the use and application of LCGMM with an emphasis on how this method differs and compliment the GCM methods introduced and developed in chapter 3 to 5, providing a further tool to investigate population heterogeneity in developmental trajectories of continuous outcomes.

Box 4. *Summary of key points from Chapter 6: Association between latent body mass index (BMI) trajectories and cardiometabolic risk markers in mid-adulthood*.

7. DISCUSSION

7.1 Background and overview of thesis aims

There is increasing interest in modeling developmental trajectories that may provide clues as to the impact and timing of early-life exposures that influence the development of important chronic diseases. In relation to cardiometabolic research, understanding how risk factors change across the life course and being able to investigate determinants associated with deviations from 'normal' or 'healthy' trajectories is important because the development of CVD and comorbidities such as obesity, hypertension, dyslipidaemia, and T2DM, have their origins in childhood, and tend to manifest over time. By modeling trajectories of cardiometabolic risk factors across key stages of the life course that precede the clinical presentation of the disease (childhood, adolescence, young and mid adulthood), it is expected that prevention, screening, and treatment of risk factors could be improved or optimised to prevent or delay adverse cardiometabolic outcomes later in life. However, modeling trajectories of continuous risk factors across the life course for chronic disease outcomes remains under used in many settings owing to a number of specific and perceived technical challenges.

The overall aim of this thesis has been to examine GCM approaches for the analysis of non-linear continuous biological response data that arise from long-term, cohort-sequential, or accelerated longitudinal designs spanning different developmental periods (childhood, adolescence, and adulthood). The specific contribution was to review, develop, formulate, and apply a range of GCM approaches to analyse and model the life course trajectories of a number of important cardiometabolic risk factors and outcomes in a well-studied European cohort, the YFS. For this cohort, data on metabolic risk factors was collected on up to eight occasions over 31 years from childhood to adulthood among a large number of participants that were initially representative of Finnish children. Specifically, this work focused on the technical

aspects of growth curve methods that pertain to modeling non-linear age-related trajectories of continuous response variables in study designs where participants from unique birth cohorts contribute to different and, to some degree, overlapping segments of cohort member's life course trajectory.

This dissertation addressed the specific research aims identified in section 1.1 by:

Aim 1. Reviewing a number of existing GCM approaches, their strengths, weakness and identifying areas for further methodological development or extension for the analyses of continuous outcomes collected across decades in long-running prospective cohort studies (Chapter 2).

Aim 2. Tailoring and extending growth curve modeling theory to develop a novel analytical tool for epidemiologists, the BHPR method, which may help inform prevention and intervention strategies by allow the identification of the point in the life course at which participants from two identified groups start to diverge in their non-linear trajectories of continuous modifiable risk factors (Chapter 3).

Aim 3. Applying the statistical methods reviewed in Aim 1 and developed in Aim 2 to examine specific aims important in developmental cardiometabolic research using data from YFS in four original manuscripts (Chapters 3 to 6).

7.2 Specific approaches and their importance

More specifically, the first three original studies (Chapters 3 to 5) considered person-based GCM approaches (1-4) to model individual trajectories of important continuous cardiometabolic risk factors using repeated measure data collected at up to eight time points in the YFS study, spread across the developmental period from childhood to adulthood. In these chapters, the grouping variables of interest were included as determinants or predictors, of between-person differences in the continuous response

over time (5, 6), a situation relevant in a variety of observational study settings (7) . Depending on the specific research question in each study, these chapters considered different types of grouping variables, commonly encountered by epidemiologists in practice. This included the stratification of participants into groups of interest related to (1) pre-existing individual characteristics (i.e. high/low individual genetic risk scores, Chapter 3) that may influence the developmental response trajectory over time, or (2) the realisation (or not) of a dichotomous heath outcome at a later point in time for some of the participants (i.e. those who did or did not develop T2DM in adulthood, Chapter 4), or (3) the change in response status between two time points (i.e. change in BMI status between childhood and adulthood across four groups of clinical relevance, Chapter 5). In all examples, the categorical grouping variable was used to divide the longitudinal data into two or more non-overlapping, mutually exclusive subsets of data with the aim to determine when differences between these *a-priori* identified subsets occur (8-10).

These types of approaches are important because grouping based on participant-level characteristics (e.g. sex, smoking status, etc.) allows researchers to test whether having the characteristic modifies the average level, the initial level, or the shape of the response variable over time (11). Moreover, when the stratification is based on the development of a distal outcome of clinical importance or change in response status, researchers can use these GMC approaches to examine the trajectories of the response profiles between groups, to determine if, and when, participants exhibit similar or different response trajectories. This allows the determination of the point or ages in the life course when risk factor trajectories diverge between the different groups, which are often of clinical interest for the researcher(12-14).

In these three chapters, proposed approaches covered a range of possible model parameterisations that the researcher may consider to adequately represent the non-linearity in response within-person over time. Chapter 3 illustrated the empirical determination of the within-person response shape in a classical non-Bayesian

multilevel framework, with genetic risk score groups used as level-2 predictors of between-person differences in higher-order polynomial age terms describing individual lipid trajectories in the YFS cohort from age 6 to 49 years (15-17). In Chapters 4 and 5, the Bayesian formulation and estimation of a piecewise linear random effect multilevel model was developed and applied to model within- and between-person trajectories of BMI from childhood to adulthood (18-20). In these models, the change points were estimated from the data and allowed to vary between participants (i.e. random change points), mapping onto known aspects of BMI development from childhood to adulthood. In this chapter, this developed modeling approach was applied to the YFS data to determine the age at which two groups (those with, and those without adult T2DM) who share the same population-average BMI trajectory until a certain point in time, go on to separate BMI paths later in the life course. In chapter 5, an alternative formulation of the Bayesian hierarchical piecewise modeling approach developed in Chapter 4 was proposed to allow the estimation and comparison of average trajectories of BMI in the YFS according to four adiposity groups to enhance understanding of the developmental BMI patterns of participants in four clinically important groups.

The fourth original chapter, Chapter 6, took a different approach from the other three chapters by considering Latent Class Growth Mixture modeling (LCGMM), a state of the art group-based approach to GCM (21-24). Rather than analysing pre-assigned groups, this method allows latent classes of unobserved trajectory patterns to be identified within a population. Despite its promising features to analyse longitudinal data, this recent approach is technically quite challenging and remains largely underused by epidemiologists with long-term cohort sequential data (25, 26). This chapter illustrated the use of the LCGMM approach in the non-Bayesian framework with a polynomial parameterisation for the within-person model to investigate the heterogeneity in developmental patterns of BMI between age 6 and 49 years in the YFS.

The various statistical models considered in these four chapters were all formulated and fitted through the R interface, either using the `nlme, lmer,` or `lcmm` mixed and growth mixture modeling packages (Chapters 3 and 6) or using `rjags` and `r2jags` (Chapters 4 and 5). The latter two packages provide a linkage between the graphical and statistical capabilities of `R` and the MCMC sampling of `JAGS` (a clone of the Bayesian Analysis Using Gibbs Sampling program, `BUGS`). The Bayesian estimation method allowed complex models to be fitted, in particular the piecewise growth models with two linear segments and random change points in Chapters 4 and 5, which could not have been estimated using popular likelihood–based software.

The narrative of this thesis is recounted in the following section, where the statistical approaches, the major findings of individual studies (chapters 3 through 6), and implications for cardiometabolic health research are considered. As strengths and limitations of each study were identified and discussed at the end of each chapter, they will not be reiterated here unless they are central to the topics covered.

7.3 Summary of study findings and implications

Chapter 3 demonstrates the use of hierarchical higher-order polynomial specification of GCM to model response profiles that have complex non-linear forms overtime when no prior knowledge or existing biological theory can be used to inform the modeling over the time period of interest(27, 28). This chapter illustrates complex model building strategies to model of individual response trajectories and to investigate both the longitudinal and cross-sectional associations between the response and categorical or continuous risk factors. In this chapter, the polynomial GCM approach was applied to analyse blood lipid trajectories in the YFS between 3 and 49 years of age, primarily to examine whether the polygenic effect of a predisposition for abnormal levels of circulating lipids was present at all ages, or whether it varied by age. The rationale for this study was that despite an increasing number of longitudinal and genetic studies in the past 10 years, the patterns of

lipoprotein trajectories across the life course, and how genetic factors influence these long-term patterns, remain poorly understood (29, 30). In this application, the polynomial specification of MLM growth curve models allowed the empirical determination of the shapes of individual longitudinal lipid profiles from childhood to adulthood in the YFS. Moreover, it allowed determining if identified risk alleles collectively predict lipid trait levels at all ages or whether they modify the intra-individual progression of serum lipids as people age. This application illustrated key aspects of this advanced modeling technique, including the creation of interaction terms with all higher order polynomial terms to adjust for time-varying covariates or birth cohort effects, and strategies to address potential heterogeneity and within-person autocorrelation of error. The main clinical finding from this study was that for LDL-C, HDL-C and triglycerides, the polygenic effects of GWAS-associated alleles were present at all ages in the YFS, even in early childhood, and strongest for LDL-C. In addition, results suggested that for HDL-C levels, the combined genetic effect became weaker as participants aged, and conversely for triglyceride, stronger in males with age. The results of this study have important clinical implications, since they suggest that modifiable lifestyle factors may become important determinants of HDL-C levels in adulthood and that males with high genetic risk for high triglycerides levels may not respond as well as females to lipid-reducing lifestyle interventions. While complex, the modeling approach used in this study provided crucial insight on the influence of genetic risk factors in long-term lipid profiles. Since the functional form of within-person change in response over time is determined form the data itself, this approach is particularly suited to explore the development of cardiometabolic factors for which the typical development is not well characterised over the life course, when the main interest is to investigate the effect of determinants of interest on outcome trajectories.

In Chapter 4, a novel individual-based growth modeling approach (BHPR) was formulated and developed to determine how and when two or more groups of participants diverge in their curvilinear trajectories of continuous outcomes in long-

term observational studies. In this chapter, the motivation for the development, and subsequent application of this method was the lack of existing methods dedicated to between-group trajectory divergence analysis in longitudinal studies. Specifically in the YFS, the rationale for developing this tool was the ability to be able to refine the estimate of the age at which developmental BMI trajectories diverge between participants who do and do not develop adult T2DM (31). The developed approach considers segmented regression models with random change points to summarise and approximate individual BMI response profiles from childhood to adulthood, in line with biological knowledge from several decades of developmental research (32, 33). These complex models are formulated and fitted in the Bayesian framework to circumvent estimation issues often encountered with traditional maximum likelihood methods. To investigate trajectory divergence mechanisms between groups of participants, the proposed modeling approach sequentially introduces group differences in each trajectory parameters related to identified aspects of the age-related change in BMI profiles, before choosing the best fitting model among the set of candidate models with the aid of Bayesian model selection tools. In addition, this chapter illustrates critical aspects of the proposed modeling technique, including the inclusion of cohort effects, time-varying covariates, and issues related to prior specification and sensitivity. Through simulation, this chapter demonstrates the significance of the developed approach for the chronic disease research community, by showing that the BHPR is less dependent of sample size and provides a more accurate estimate of the age at which the change occurs between group of participants, compared with more traditional modeling approaches. From a clinical perspective, when applied to the BMI and T2DM data in the YFS, the developed approach revealed the existence of a ‘critical window’ for weight management intervention in the years around puberty that may help inform strategies targeting the incidence of overweight-related T2DM. Although developed for a two-phase developmental process, this statistical tool could easily be extended to investigate between-group trajectory divergence in non-linear response of more complex shapes by defining a three or more piece functional form for the level-1 model. A three-piece

BHPR model with two random change points, would be particularly suited to model early life BMI trajectories, allowing the direct modeling of two crucial individual BMI milestones in early life, the infancy peak and the adiposity rebound.

Chapter 5 illustrates a different application of the BHPR formulated in Chapter 4. Rather than trajectory divergence between groups, this chapter demonstrates how a slightly modified formulation of the linear-linear segmented model with random change points enables comparison of curvilinear response profiles between more than two groups. Specifically, this modified BHPR approach was used to model individual life course BMI profiles in the YFS cohort, and to compare them between four groups of clinical interest, determined *a-priori* based on participants' change in BMI status between childhood and mid-adulthood (34). The rationale for this study was to build on and refine previous findings from a large multicentre study that found, using a limited number of time points, overweight or obese children who became non-obese in adulthood had a similar risk for adult cardiometabolic outcomes than those who had never been obese (34). Although these findings provide hope to paediatric care providers that a sustained change in BMI can normalise cardiometabolic risk among obese children, this multicentre study was only able to pinpoint that this group had transitioned status at some time over the 23-year period between child and adult measurement – limiting the broader implications of their findings to the public health and clinical setting. Using the full set of repeated BMI data in the YFS, Chapter 5 used the novel modeling approach developed in Chapter 4 to refine the understanding on the dynamics of sex-specific BMI developmental in each of the four groups considered in the multicentre study. This chapter illustrated how Monte Carlo Markov Chain techniques can be used to compare BHPR trajectory parameters from two given growth curves, by sampling the marginal posterior density of the difference in growth parameters between groups (35, 36). Applying this modified BHPR approach to data from the YFS, the timing of BMI differences in early life between those who do or do not maintain high-BMI levels, or who develop incident obesity, from childhood to adulthood was determined. The findings from this trajectory

modeling application suggest that efforts to influence BMI trajectories that lead to adult obesity should begin early in life, ideally before age 6 years, with the existence of a 'critical window' in adolescence for secondary prevention. Significantly, the BHPR approach provided increased clarity to paediatric care providers on the critical age windows when prevention, screening, and intervention for elevated BMI levels or rates of change could be implemented – which was the major limitation of the previous multicentre study(34).

Chapter 6 introduces, investigates, and formulates GCMs in a different statistical paradigm, which is becoming increasingly popular in the trajectory modeling in psychology and cognition research, but remains largely underused by the public health community, especially in the context of analysing data from long-term cohort sequential studies (37-39). In this chapter, LCGMM, a group-based GCM trajectory modeling approach, was used to reanalyse the developmental BMI trajectories YFS cohort from another perspective. Unlike the modeling approaches used in the three preceding chapters, the LCGMM used in this chapter does not rely on pre-determined groupings to categorise and contrast different subgroups of interest in the population. This recent approach thus decreases the potential for misclassification issues, as well as the loss of information related to discrete categorisation of longitudinal profiles (22, 23). This study was motivated by findings in Chapter 5, where model checks suggested unaccounted for variability in the model residuals. In contrast to the BHPR modeling approach used in Chapter 5, LCGMM allows the clustering of participants based on their entire response profiles. It was used to investigate the underlying heterogeneity that may exist in the developmental trajectories of BMI in the YFS with the aim of identifying common BMI patterns within this population and how these patterns predict important adult cardiometabolic risk phenotypes. Chapter 6 considers issues pertaining to model building in LCGMM and illustrates the different steps to build these complex trajectory models. In particular, it shows how to fit and compare various LCGMMs before choosing a final model, considering solutions with increasing number of classes, and strategies to specify the variance-covariance of

growth parameters and within-class heterogeneity. In addition, it presents strategies to to evaluate model goodness-of-fit and to characterise the discrimination ability of estimated classes. Applied to the longitudinal BMI data in the YFS, this modeling approach enabled six distinct BMI trajectories to be identified, including a subgroup with a progressively overweight trajectory that could not be discriminated from the truly normative BMI trajectory group in Chapter 5. Two groups with distinct trajectories leading to adult obesity were also distinguished: one of early onset, stabilising obesity and another of later onset, worsening obesity. This reanalysis of the long-term BMI profiles in the YFS provided increased clinical granularity to the previous analyses of these data was part of the multicentre. The LCGMM used in Chapter 6 revealed that those with higher and increasing trajectories of BMI were generally associated with increased cardiometabolic risk phenotypes in middle age compared with those who remained on an ideal BMI trajectory throughout their life course. Although completely reversing elevated BMI later in life may decrease the risk for hypertension, dyslipidaemia and T2DM, the results of this study, in line with recent findings in the literature, suggested that the effect of deleterious BMI status in early life on cIMT might not be reversible even with normalisation of weight status in later life (40). The results obtained through this alternative approach to GCM analysis are important to paediatric care providers because the previous approach (prone to misclassification) used in the multicentre study suggested no residual risk of elevated child BMI (34). This Chapter illustrates the application of this complex method in practice, and highlighted its potential as an additional tool to analyse the heterogeneity in long-term trajectories among given populations

7.4 Future directions

The specific research findings obtained using the reviewed, applied, and developed statistical approaches in this thesis have implications for future cardiometabolic research, as well as for prevention and intervention of adverse cardiometabolic phenotypes in children, adolescents, and young adults. Based on findings from this

thesis, future research focusing on the trajectories that predispose to later cardiometabolic disease ought to investigate the following specific areas:

- **Importance of individual BMI trajectory parameters in the prediction of later cardiometabolic outcomes.** In Chapter 4, since the focus was on the age at divergence in BMI trajectories between participants who do and do not develop adult T2DM, the association between the longitudinal BMI profiles and actual adult T2DM outcome was not investigated; rather, the outcome of T2DM was used to stratify the participants within two groups of interest. Future studies should investigate if faster rates of BMI increase estimated form the BHPR approach (i.e. greater velocity) in childhood, adulthood or older age at the change point (i.e. greater random deviations from population average in each growth parameters) associate with an increased risk for developing adult T2DM. Indeed, compared to existing risk prediction models commonly used in cardiometabolic research, which often include a single measure of risk in childhood or early adulthood (i.e. single lipid or single BMI measure) to predict later adult outcome(41), the addition of these individual trajectory parameters obtained by BHPR (or by traditional growth modeling techniques, such as the polynomial GCM in Chapter 3) may provide more accurate predictions of CVD outcomes.

- **Identification of the socio-demographic, biological and behavioural factors associated with distinct developmental BMI profiles**. In the YFS, LCGMM modeling used in chapter 6 suggested the existence of six distinct BMI trajectory classes. Being able to identify predictors of class membership, and thus of distinct BMI life course patterns, could help identify those at increased risk of developing adverse BMI levels at different age periods. Characterising those participants who managed to completely normalise their BMI levels after being obese/overweight in youth is also of potential interest from a public health perspective. Determining the best times to intervene and the most appropriate strategies for intervention

will be an important challenge towards reducing the prevalence of overweight and obesity globally.

- **Age-dependent genetic effects on circulating lipid levels**. Some of our findings in Chapter 3 are difficult to interpret clinically and will need further investigations. Notably, we found that the combined genetic effect of 24 common variants on circulating triglycerides becomes stronger in men as they age. This is conflicting with the expected pattern, which is be that genetic effect remains constant or becomes weaker with age (as we found for HDL-C), with environmental, lifestyle and behavioural factors such as diet, smoking, and physical activity becoming more important determinants of adult lipid levels. While the increase in the means and variances of most lipid parameters as humans age has been well established in the literature, how much of that age dependency is due to increases in environmental or genetic variance, or both, is still largely undetermined[1]. For other complex traits (such as BMI and systolic blood pressure) preliminary studies suggest that, over time, while the contribution of genes to a phenotype remains relatively constant, environmental exposures tend to increase the phenotypic variance, contributing to between-person differences in trait [2]. Thus, it is possible that the accumulation effects of environmental exposures (i.e. diet, physical activity, or smoking) increase the phenotypic variance of lipids over time, making it more difficult to detect genotype-phenotype associations. Recent linkage analyses and GWAS studies, for a few lipids, have found evidence that age was a modifier of genetic associations between specific loci and HDL-C and LDL-C levels [1, 3 4]and that the genetic architecture of lipid traits changes over time [5], but no study has yet considered combined genetic risk score with multiple loci. More research is indeed needed to understand the implications of our findings on triglycerides profiles. More research is needed to fill these gaps.

- **Time-varying gene-environment interactions predicting important cardiometabolic outcomes**. In chapter 3, it is interesting (and perhaps a little intriguing) that BMI change between childhood and adulthood did not seem to modify the genetic effect on adult lipid levels in our study sample. Indeed, despite having identified multiple loci robustly associated with plasma lipids and extreme lipid phenotypes, most recent GWAS studies also report that common genetic variants explain <12% of variation in lipid traits. As adiposity is also an important determinant of plasma lipoproteins, particularly plasma TGs and HDL-C concentrations, several studies have hypothesised and demonstrate that interactions between genes and adult clinical phenotypes may contribute to this unexplained heritability[6]. No study however, has looked at genetic effect modification as a function of longitudinal change in BMI across the life course. The apparent lack of gene-environment effect in this study could be due to other time-varying environmental variables likely to mediate the effect modification of longitudinal change in BMI (such as exercise and diet, that we did not consider in our analyses). In general, the gene-environment effect on lipid levels reported in the literature is equivocal, and gene–gene and gene–environment interactions, gene–environment correlations, and epigenetic mechanisms remain active areas of research in the field[6], and warrants more research.

- **Relative impact of severity of obesity, duration of obesity, or a combination of both on future cardiometabolic outcomes**. In Chapter 6, although the identified latent classes of BMI trajectories provide an idea of which aspects of BMI development across the life course might influence risk for each of the studied outcomes, the LCGGM modeling approach does not allow the researcher to formally investigate the associations between obesity duration or severity and later cardiometabolic outcomes. More research is needed in this area to determine what aspect of obesity best predicts adult cardiometabolic outcomes such as T2DM, high cIMT, dyslipidaemia and hypertension. Teasing apart the effect of

obesity duration from the effect of obesity severity on later outcomes is likely to require further methodological development in growth modeling techniques.

- **Life course trajectories of BMI and blood lipids of the metabolically healthy obese**. Further research is needed to investigate the developmental patterns of BMI among obese adults who do not display the typical metabolic disorders associated with obesity (i.e. metabolically healthy obesity (42)), and how their cardiometabolic risk profile compares with those of obese participants who have developed other cardiometabolic complications. In particular, it would be useful to determine whether distinct developmental BMI profiles exist within this subgroup of metabolically healthy obese participants, to help determine if change in adiposity at different critical periods in the life course can help identify a metabolically healthy obese phenotype. This is important since it may provide additional insight for the identification of critical windows in the development of obesity and implications for future cardiometabolic risk.

- **Life course cardiometabolic risk factor trajectories and clinical endpoints.** It should be noted that, with the exception of T2DM, the other risk factors considered throughout this thesis are risk factors for CVD. It would be important to replicate these analyses in samples where clinical endpoints such as confirmed CVD events or deaths are available.

From the methodological point of view, although this thesis adds to the existing GCM literature for modeling longitudinal continuous outcomes in multi-cohort observational studies, more work is necessary in order to apply some of the more advanced GCM approaches to complex data sets with multiple groups and additional covariates. Growth modeling is an active and fast growing area of research, and the strength and the flexibility of GCMs provide opportunities for testing theoretically derived hypotheses in ways not previously possible. For example, it provides epidemiologists an opportunity to gain a better understanding of individual

differences in developmental change. There are many directions that can be taken to further enhance our understanding of the benefits of these models and to make them more useful in practice. This thesis identified a number of important remaining methodological issues that ought to be addressed in future GCM research. This includes:

- **GCMs with binary or discrete outcomes.** The limited available literature on the treatment of longitudinal binary and ordinal outcomes in the CGM framework motivates further research (5). With the growing popularity of GCM modeling as the preferred analytical choice to test hypotheses related to individual differences in responses collected across the life course, significant resources have accumulated to instruct researchers in the application of GCMs with longitudinal continuous and count outcomes (6) (43-45). With the exception of three works (37, 46, 47), the discussion regarding binary and ordinal outcomes in GCM is by comparison, quite limited. In cardiometabolic research, such models may be particularly useful if the longitudinal outcome of interest is the presence vs. absence of early atherosclerosis, T2DM, or metabolic syndrome across the life course and the rationale to investigate population heterogeneity in the development of these CVD outcomes.

- **Non-parametric GCMs**. The standard GCMs models considered in this thesis assume a parametric relationship between time and the response variable and covariates, however, this assumption is not always met and can lead to restrictive and inaccurate models (5). The recognition of the limitation of parametric models with practical data in biomedical research calls for the development of innovative non-parametric GCMs methods, which are more flexible in the shape of the underlying function than their parametric counterparts. These non-parametric approaches may be more adept at capturing the true underlying change pattern than existing parametric approaches, when the shape of the function over time is complex. At present,

frequentist current non-parametric GMC approaches, including methods based on polynomial kernel smoothing, regression splines, and smoothing remain underdeveloped and limited in their abilities. Bayesian framework is appealing for its ability to handle such complex models (i.e. varying sample sizes, non-normal outcomes and within subject correlation) but the implementation of such methods is not well studied and literature is relatively sparse (17, 48, 49).

- **Modeling multiple outcomes in GCM analyses**. The multivariate GCM approach allows flexible modeling of relationships between two or more longitudinal response variables such as correlated residuals over time and regressions among different outcomes over time. Currently, GCM models for correlated, coupled, and conditional change remained under developed. In some situations, rather than considering them separately, the researcher may be interested in jointly modeling two outcome variables (e.g. parallel growth models – change in multiple CVD risk factors as a function of time, to determine if a positive change in lipid levels would occur in response to a reduction of say, BMI). Or it might be of interest to model simultaneously the same outcome measured on different (identifiable, nonexchangeable) but related individuals (e.g. change in spouse/sibling BMI as a response to an obesity intervention). At present, the three main approaches to investigate multivariate relationships among developmental functions (i.e. Multiple Correlated Growth Curves - estimation of covariation among levels, slopes, and time-specific residuals, Curve-of-Factors - LGM based on measurement model, and Factor-of-Curves - evaluation of factor structure of covariance among levels and slopes) remain difficult to apply in practice (5, 50) and are mostly implemented in the SEM framework. These complex models, including multivariate change point models, are likely to be easier to estimate in the Bayesian framework but this area needs to be extended (51, 52).

- **Estimating statistical power for GCMs.** Power and sample size analysis is a critical aspect in study design. The power of a statistical test is usually defined as the probability that the test would reject a false null hypothesis. The main purpose of a prospective power analysis is to plan the sample size for a future study. A retrospective power analysis is conducted after data collection and analysis, the purpose of which is to determine if the obtained effect size is conclusive. Although GCMs have become among the most powerful tools in facilitating the analysis of change in the past 50 years, discussions on the determination of sample sizes or power analysis of these models (both in the SEM and MLM framework) are still relatively rare, with a few exceptions (53, 54) Some studies have investigated the power of LGC models by using the chi-square difference test or the likelihood ratio test from different perspectives (55, 56). In addition, some power analysis methods available for multilevel or mixed-effects models can be used to analyse certain types of GCMs (57, 58). However, procedures do no exist for more complex model structure. The ones that do exist often require the use of several pieces of software to compute power as they are based on chi-square difference test for covariance analysis. The two-step, two-software approach makes power analysis cumbersome and hinders the broader adoption of the method (54).

7.5 Conclusions

This thesis has contributed to the body of evidence examining modern statistical approaches suitable to (1) model complex non-linear trajectories of continuous risk factors in long-term cohort-sequential studies at the individual or group level, (2) identify distinctive profiles in these trajectories that may exist within a population sample, or (3) investigate critical phases of change in these trajectories that may be related to higher risk of a distal health outcome. In particular, this thesis has focused on growth modeling methods and their extensions, an expanding framework that has a tremendous amount to offer to a broad array of developmental research endeavours

and represent a powerful set of tools to better understand between-person differences in individual change over time. The focus of this thesis has been on how these methods can help gain insight in the development of a number of important cardiometabolic risks and outcomes. More broadly, concepts and methods reviewed, developed and applied throughout this thesis are relevant to research in other chronic diseases that typically manifest in later life, such as cancers.

Studying long-term trajectories of risk factors for future disease and demonstrating the influence of these trajectories on later outcome brings several analytical challenges. Therefore, it likely that a combination of approaches will be necessary to unravel the complexities and nuances observed in life course research, such that the maximum information can be obtained from the available data. Each of the approaches illustrated and discussed throughout this thesis have their limitations and strengths, with the choice of methodological approach depending upon the nature of the available data and the specific research questions.

To this end, this work has developed a novel GCM approach that allows the mechanisms of divergence in non-linear trajectories between groups of participants in long-running cohort studies to be characterised, and has demonstrated how different GCM approaches provide unique data that might help inform, refine, and improve prevention and intervention applications in clinical and public health settings.

APPENDICES

Appendix 2.1. Custom-written self-starting non-linear functions in R allowing the estimation of 4 parameters Gompertz and 5 parameters Richards Growth Curve Models, respectively.

```
#####################################################################
### Function to estimate a 4 parameters Gompertz Growth Curve model
#####################################################################

SSGompertzGR <- selfStart(y ~ g0 + g1*exp(-exp(-a1*(x-a0))),
                          function(mCall,data,LHS) {
                            xy <- sortedXyData(mCall[["x"]], LHS,
data)
                            if(nrow(xy) < 4) {
                              stop("Too few distinct x values to fit
a GompertzGR model")
                            }
                            xy["y"] <- cummax(xy["y"])
                            g0 <-
stats:::NLSstLfAsymptote.sortedXyData(xy)
                            g1 <-
stats:::NLSstRtAsymptote.sortedXyData(xy)-g0
                            xy[["z"]] <- log(-log((xy[["y"]]-
g0)/g1))
                            cs <- coef(lm(z ~ 1+x,data=xy))
                            a1 <- -cs[2]
                            a0 <- cs[1]/a1
                            setNames(c(g0,g1,a0,a1),
                                     mCall[c("g0","g1","a0","a1")])
                          },
                          c("g0","g1","a0","a1"),
                          function(x,g0,g1,a0,a1) {})




#####################################################################
### Function to estimate a 5 parameters Richards Growth Curve model
#####################################################################

SSRichardsGR <- selfStart(y ~ g0 + g1/(1+a2*exp(-a1*(x-a0)))^(1/a2),
                          function(mCall,data,LHS) {
                            xy <- sortedXyData(mCall[["x"]], LHS,
data)
                            if(nrow(xy) < 5) {
                              stop("Too few distinct x values to fit
a RichardsGR model")
                            }
                            xy["y"] <- cummax(xy["y"])
```

```
                            a2 <- 1
                            g0 <-
stats:::NLSstLfAsymptote.sortedXyData(xy)
                            g1 <-
stats:::NLSstRtAsymptote.sortedXyData(xy)-g0
                            xy[["z"]] <- log( ((g1/(xy[["y"]]-
g0))^a2 - 1)/a2 )
                            cs <- coef(lm(z ~ 1+x,data=xy))
                            a1 <- -cs[2]
                            a0 <- cs[1]/a1

setNames(c(g0,g1,a0,a1,a2),mCall[c("g0","g1","a0","a1","a2")])
                          },
                          c("g0","g1","a0","a1","a2"),
                          function(x,g0,g1,a0,a1,a2) {})
```

Appendix 2.2. Illustrative annotated R code showing custom non-linear functions to estimate additive and multiplicative Gompertz growth curve models on simulated data from sequential cohort design.

```
#####################################################################
### Simulate Gompertz growth data with random effects for the 4
parameters ##################################################

## t0=age 3 (initial age in the cohort)

rm(d)
library(mvtnorm)

#############################################
######### 4-parameters Gompertz model ########
#############################################

# 4 parameters in the function: g0","g1","a0","a1"
# fits : y ~ g0 + g1/(1+exp(-a1*(x-a0))
#d <- local({
 # x <- seq(3,50,3)
 # y <- SSGompertzGR(x,15,30,9,0.2)+rnorm(length(x),0,0.2)
 # data.frame(x=x,y=y)
#})
#plot(y~x,data=d,pch=16,col="firebrick")

# equivalent to :
#param<-c(15,30,9,0.2)
#x <- seq(3,50,3)
#y<-param[1]+ param[2] /(1+exp(-param[4] *(x-param[3])))
#plot(y~x)

##################### Simulate sparse-cohort sequential data with
INDEPENDENT RANDOM EFFECTS for each of the Gompertz curve parameters
#################################

nid=100 # 100 subjects
baseline <- c(3,6,9,12,15,18)# ages at baseline
follow <- c(0,3,6,9,12,21,27,31)# up to 7 follow-ups  per person ==>
maximum of 8 repeated obs. per person

V <- diag(c(2,4,2,0.01)^2)# variance-covariance matrix for the
random effects (i.e. 4 gompertz param)
re <- rmvnorm(nid,sigma=V)


d <- data.frame(id=factor(1:nid),
```

```
                Baseline=sample(baseline,nid,replace=T),
                re=re)[rep(1:nid,each=length(follow)),]
param<-c(15,30,9,0.2) # Gompertz parameters
sigma <- 0.9 #error
d$age <- d$Baseline+follow
d$mu <- with(d,(param[1]+re.1)  + (param[2]+re.2 )/(1+exp(-
(param[4]+re.4) *(age-param[3]+re.3)))) # generate mu
d$bmi <- rnorm(nrow(d),d$mu,sigma) # with residual error

library(ggplot2)
# Plot simulatedgompertz BMI data with smoother:
ggplot(d,aes(x=age,y=bmi))+geom_point()+geom_smooth()
# Show indivudal plots
ggplot(d,aes(x=age,y=bmi,group=id,colour=id))+
  geom_point(size=1.2)+
  geom_path()

# write csv file with simulated data
# sim.csv contains simulated data with 8 observations per subj
write.csv(d, file = "sim.csv")

#####################################################
### 1- Fit custom-build Gompertz model function to simulated cohort-
sequential data ###################################################

library(dplyr)
d <- read.csv('sim.csv', header=TRUE,na.strings='.') %>%
 # everyone has over 5 BMI obs in this situation
  group_by(id) %>%
  mutate(nobs=n()) %>%
  filter(nobs >=5) %>%
  ## Select a subset of columns
  select(bmi,age,id) %>%
  data.frame
d$id <- factor(d$id)
head(d)
str(d)

## =>everyone has 8 BMI observations. Due to different ages at
baseline, the observations stretch across different periods across
the life course

## Custom-written Self-starting function to estimate 4 parameter
Gompertz model##
SSGompertzGR <- selfStart(y ~ g0 + g1*exp(-exp(-a1*(x-a0))),
                          function(mCall,data,LHS) {
                            xy <- sortedXyData(mCall[["x"]], LHS,
data)
                            if(nrow(xy) < 4) {
```

```
                                stop("Too few distinct x values to fit
a GompertzGR model")
                              }
                              xy["y"] <- cummax(xy["y"])
                              g0 <-
stats:::NLSstLfAsymptote.sortedXyData(xy)
                              g1 <-
stats:::NLSstRtAsymptote.sortedXyData(xy)-g0
                              xy[["z"]] <- log(-log((xy[["y"]]-
g0)/g1))
                              cs <- coef(lm(z ~ 1+x,data=xy))
                              a1 <- -cs[2]
                              a0 <- cs[1]/a1
                              setNames(c(g0,g1,a0,a1),
                                       mCall[c("g0","g1","a0","a1")])
                            },
                            c("g0","g1","a0","a1"),
                            function(x,g0,g1,a0,a1) {})

# fit  Gompertz model as an NLS (non-least square model)(to get
starting values for the parameters)
fit1.0 <- nlsList(bmi~SSGompertzGR(age,g0,g1,a0,a1)|id, data=d)#
fits it for most people
fit1.0 #
fixef(fit1.0)# estimated fixed effects # if age 3 is not xpecified
as time 0 : go=23.23

# Fit gompertz as a mixed model
s <- fixef(fit1.0)

# Additive gompertz:
# random g0
fit1.1 <- nlme(bmi~SSGompertzGR(age,g0,g1,a0,a1),
             data=d,
             fixed = g0+g1+a0+a1~1,
             random = g0~1|id,
             start = c(s[1],s[2],s[3],s[4]))
summary(fit1.1)

# random g1 (upper asymtote - lower asymptote)
fit1.2 <- nlme(bmi~SSGompertzGR(age,g0,g1,a0,a1),
             data=d,
             fixed = g0+g1+a0+a1~1,
             random = g1~1|id,
             start = c(s[1],s[2],s[3],s[4]))
summary(fit1.2)

# random g0 and g1
# random g1 (upper asymtote - lower asymptote)
```

```
fit1.3 <- nlme(bmi~SSGompertzGR(age,g0,g1,a0,a1),
              data=d,
              fixed = g0+g1+a0+a1~1,
              random = g1+g0~1|id,
              start = c(s[1],s[2],s[3],s[4]))
summary(fit1.3) # neg correlation between both random effects

AIC(fit1.2,fit1.3)# as expected fit 3 clearly better

###### multiplicative random effects :
# add random inflection point : a0
fit1.4 <- nlme(bmi~SSGompertzGR(age,g0,g1,a0,a1),
              data=d,
              fixed = g0+g1+a0+a1~1,
              random = g0+g1+a0~1|id,
              start = c(s[1],s[2],s[3],s[4]))
summary(fit1.4) # neg

AIC (fit1.3,fit1.4) # better

# add random growth rate : a1
fit1.5 <- nlme(bmi~SSGompertzGR(age,g0,g1,a0,a1),
              data=d,
              fixed = g0+g1+a0+a1~1,
              random =g0+g1+a0+ a1~1|id,
              start = c(s[1],s[2],s[3],s[4]))
summary(fit1.5)

AIC(fit1.4,fit1.5) # fully multiplicative random effect model is
superior

# Fully non-linear random gompertz model : plot residuals vs. fitted
plot(fit1.5)
qqnorm(fit1.5,~ranef(.)) #normality random effects

# visualize model prediction (population and individual) for all 100
individuals (8 non missing observations each)
plot(augPred(fit1.5,primary=~age,level=0:1))
plot(augPred(fit1.1,primary=~age,level=0:1))
```

Appendix 2.3. R code illustrating syntax and different strategies to model potential birth cohort effects when fitting polynomial MLM growth curve models on data arising from cohort-sequential or accelerated longitudinal designs.

The code below assumes that LongData is a data frame with variables: y=response, age=age (centred), BaselineAge=Participant's initial age, id= participant factorial identifier (such as participant number), cohort=factor identifying cohort (i.e. year of birth), BaselineAge2=square of baseline age.

```
library(lme4)

### Using the lmer() function from package lme4:
## FIXED COHORT EFFECT MODELS:
#(1) Full interaction linear model: Each cohort can have a different
linear trend and intercept (i.e. starting level of response)
model1 <- lmer(y~age*cohort+(age|id),data=LongData)

#(2) All cohorts have the same linear slope but shift to the
intercept that vary linearly with age at baseline:
model2 <- lmer(y~BaselineAge+age+(age|id),data=LongData)

#(3) Full interaction linear model where effect of baseline age on
each growth parameter is considered to be linear:
model3 <- lmer(y~BaselineAge*age+(age|id),data=LongData)

#(4) Same model as above (3) but with a quadratic age trend:
model4 <-
lmer(y~BaselineAge*age+BaselineAge2*age+(age|id),data=LongData)

##RANDOM COHORT EFFECT MODELS:
#linear  MLM growth model with random cohort effect:
model5 <- lmer(y~age+(age|id)+(age|cohortf),data=LongData)
#Alternatively, using the lme function from package nlme:
library(nlme)
model6 <- lme(y~age,random=~1+age|cohortf/id,data=LongData)
```

Appendix 3.1. Original cohort and design of the Young Finns Study from 1980 through 2011 (Table A) and number of non-missing lipid measurements per study year in the subsample of the cohort participants considered in the present analyses (Table B).

[a ,b] Limitations in the sampling size in 1989 are due to physical examinations and blood tests collected only in one centre (Turku).

Table A

Year	No.	Age													
1980	3596	3	6	9	12	15	18								
1983	2991		6	9	12	15	18	21							
1986	2799			9	12	15	18	21	24						
1989[a]	632				12	15	18	21	24	27					
1992[b]	891					15	18	21	24	27	30				
2001	2620								24	27	30	33	36	39	
2007	2159										30	33	36	39	42	45
2011	1999											34	37	40	46	49

Table B

		Age (years)																					
		3	6	9	12	15	18	21	24	27	30	33	34	36	37	39	40	42	42	45	46	49	No. Total
Study years	1980	363	397	420	425	426	383																2412
	1983		337	364	376	375	327	263															2042
	1986			339	340	340	298	268	246														1831
	1989				56	51	47	46	44	41													285
	1992					143	119	122	119	98	100												701
	2001								299	336	359	372		362		334							2062
	2007										296	314		324		354		349		302			1939
	2011												234		262		286		301		291	289	1663

Appendix 3.2. Histograms of composite genetic risk scores (wGRSs) (Figure A) and of the number of available lipid observations per participants (Figure B).

For each lipid, green colour in histogram A denotes the lower GRS quartile (i.e. the 25th percentile); red colour, the upper GRS quartile (i.e. the 75th percentile); and white colour, the interquartile range. (i.e. the 50% of the data lying between upper and lower quartile).

Figure A

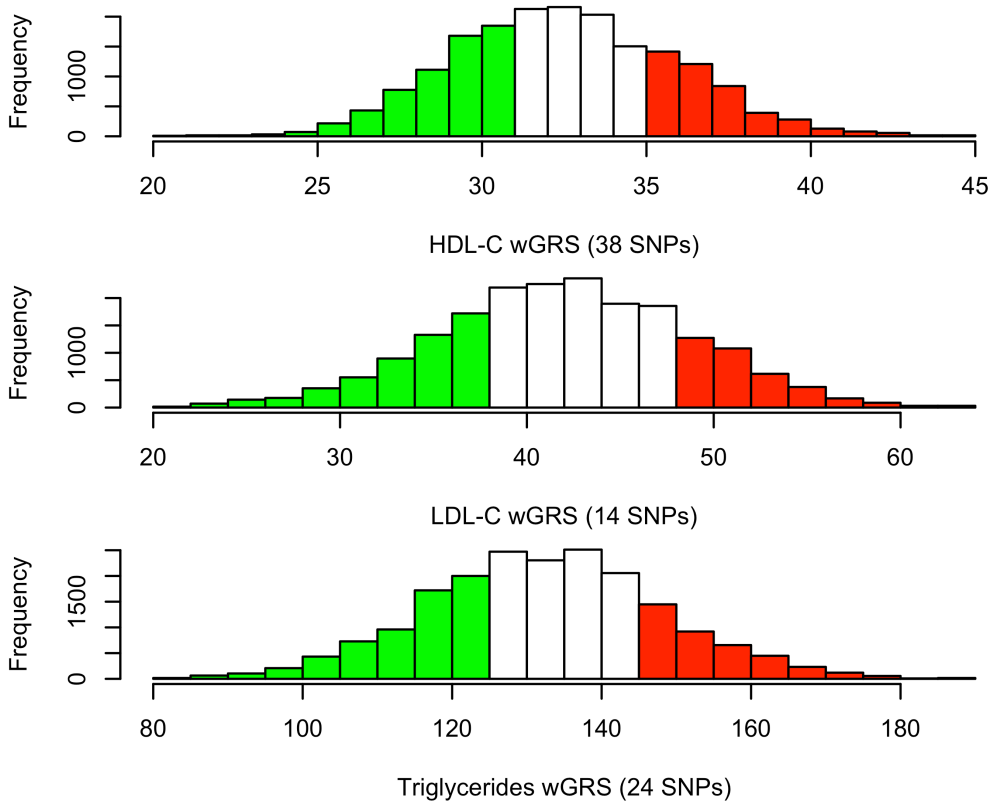
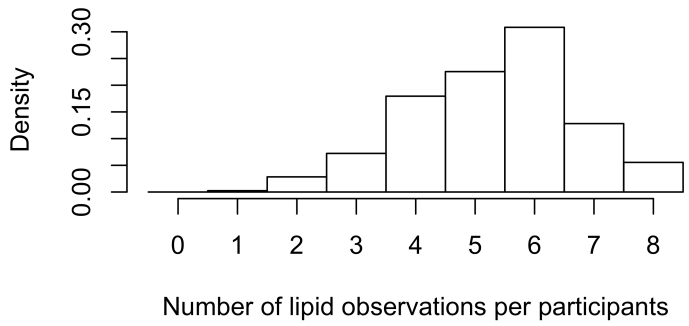


Figure B



Average number of lipid observations per participants (sd): 5.31 (1.4).

Appendix 3.3. Estimation of interaction between polygenic lipoprotein risk scores and change in BMI z-score between adulthood (ages>30 years) and childhood (ages up to 18 years) on adult lipoprotein concentration prediction.

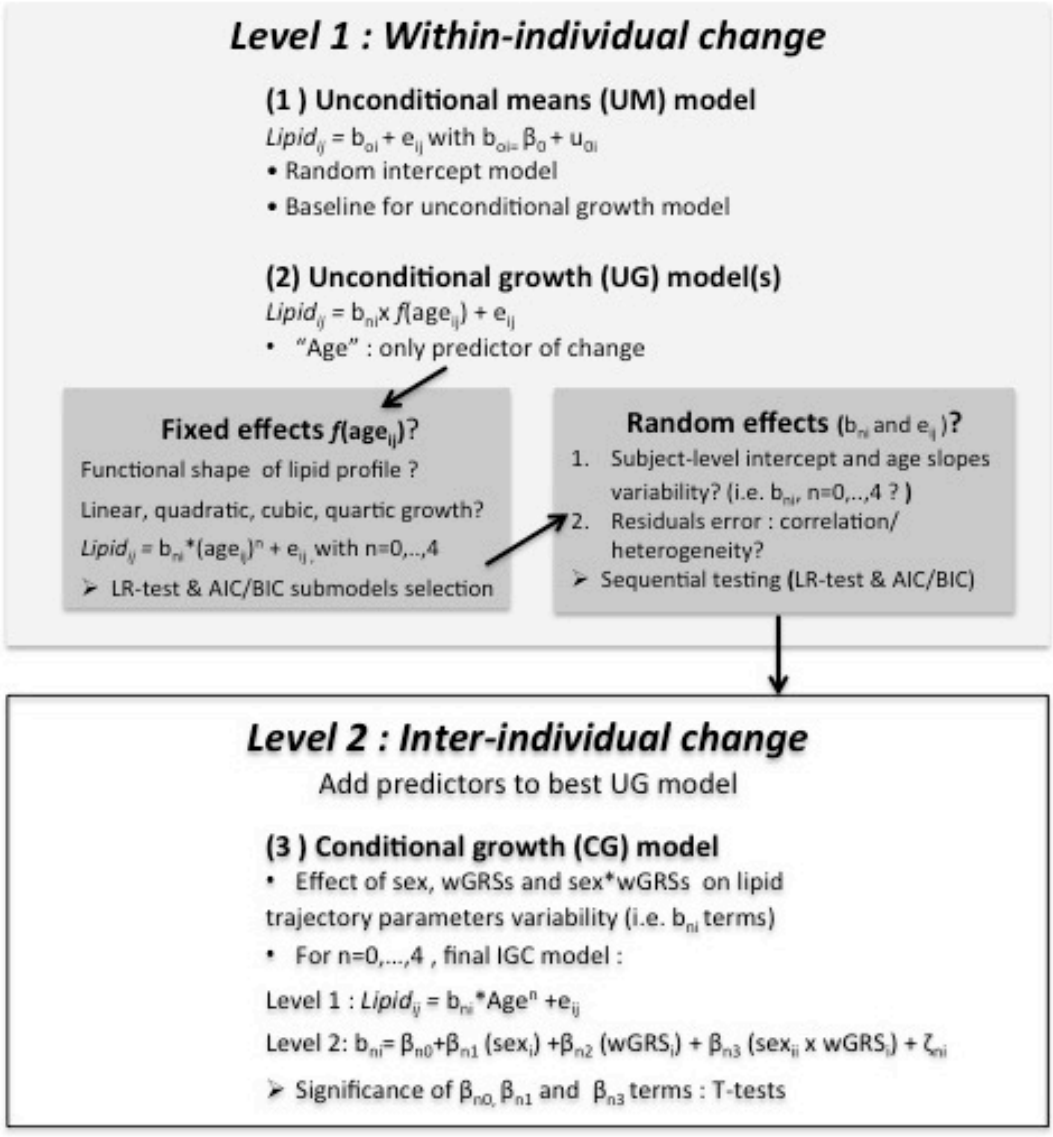
Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, wGRS-HDL; *HDLwGRS3* continuous combined genetic risk score for HDL (38 SNPs); wGRS-HDL*, LDLwGRS14, continuous* combined genetic risk score for LDL-C (14 SNPs); wGRS-TG*, TGwGRS24, continuous* combined genetic risk score for triglycerides (24 SNPs); adult.age, age of participants at latest adult follow up (2001, 2007 or 2011*),*Δ BMI, change in BMI z-score between childhood and adulthood as described in Methods section.
* For HDL-C and LDL-C, the effect of wGRSs is in mmol/L per 1-sd change in wGRS, and the effect of Δ BMI is in mmol/L per 1-sd change in Δ BMI. For triglycerides, the effect of wGRSs is in odds ratio per 1-sd change in wGRS, and the effect of Δ BMI is in odds ratios per 1-sd change in Δ BMI.

Adult LDL-C model (N=2100)	**AIC/BIC**	**β (se)*, p-val**
Model 1:	3686/3716	$\beta_{wGRS\text{-}LDL:}$ 0.029 (0.02),$p<2x10^{-16}$ *
Adult.LDL~ sex+ adult.age+ wGRS-LDL+Δ BMI + wGRS-LDL*Δ BMI		$\beta\ \Delta_{BMI:}$ 0.09 (0.01), $p=1.3x10^{-6}$ *
		$\beta_{wGRS\text{-}LDL*\Delta}\Delta_{BMI:}$-0.02(0.01), p=0.15
Model 2:	3685/3723	$\beta_{wGRS\text{-}LDL:}$ 0.20 (0.02), $p<1x10^{-16}$ *
Adult.LDL~ sex+ adult.age+wGRS-LDL+Δ BMI		$\beta\ \Delta_{BMI:}$ 0.09(0.02), $p<1.0x10^{-7}$*
Likelihood Ratio Test: Model 1 vs. model 2		
$\chi2(df=1)=2.04$ p=0.15		

Adult HDL-C model (N=2034)	**AIC/BIC**	**β (se)*, p-val**
Model 3:	486/418	$\beta_{wGRS\text{-}HDL:}$ 0.06(0.007), $p<2x10^{-16}$ *
Adult.HDL ~ sex+adult.age+wGRS-HDL+Δ BMI + wGRS-HDL*Δ BMI		$\beta\ \Delta_{BMI:}$ -0.09 (0.007), p= 0.046 *
		$\beta_{wGRS\text{-}HDL*}\Delta_{BMI:}$-0.003 (0.006),p=0.62
Model 4:	488/525	$\beta_{wGRS\text{-}HDL:}$ 0.06 (0.007), $p<1x10^{-16}$
Adult.HDL ~sex+adult.age +wGRS-HDL+Δ BMI		$\beta\ \Delta_{BMI:}$ - 0.09 (0.07), $p<2x10^{-16}$
Likelihood Ratio Test: Model 3 vs. model 4		
$\chi2(df=1)=0.24$ p=0.62		

Adult triglycerides model (N=2062)	**AIC/BIC**	**β (se)*, p-val**
Model 5: Log(Adult.TG)~sex+adult.age+wGRS-TG+ΔBM + wGRS- TG*Δ BMI BMI	2645/2876	$\beta_{wGRS\text{-}TG:}$ 1.029 (0.02),$p<2x10^{-8}$ * $\beta\ \Delta_{BMI:}$ 1.09 (0.01), $p=2.1x10^{-5}$ * $\beta_{wGRS\text{-}TG*\Delta}\Delta_{BMI:}$-1.002(0.01), p=0.3
Model 6: Log(Adult.TG)~sex+adult.age+wGRS-TG+Δ BMI	3685/3723	$\beta_{wGRS\text{-}TG:}$ 1.029 (0.02), $p<1x10^{-8}$ * $\beta\ \Delta_{BMI:}$ 1.08 (0.01), $p<1.0x10^{-9}$*
Likelihood Ratio Test: Model 5 vs. model 6 $\chi 2$(df=1)=1.17 p=0.11		

Appendix 3.4. Flowchart of the Individual Growth Curve (IGC) analyses used for modeling the blood lipid trajectories in the YFS cohort.



Appendix 3.5. List of lipid-associated SNPs used to generate the genetic risk scores for HDL-C and LDL-C in the YFS population (reported are the SNPs reference number, risk allele for the SNP, GWAS-derived effect size in md.dl^{-1}).

HDL-C (38 SNPs)				Triglycerides (25 SNPs)				LDL-C (14 SNPs)			
SNP	beta	chr/ locus	Frq (%)	SNP	beta	chr/ locus	Frq (%)	SNP	beta	Chr/locus	Frq (%)
rs4660293_A	0.48	1/PABPC4	22.1	rs10195252_T	2.01	2/COBLL1	65.2	rs4299376_G	2.75	2/ABCG5/8	20.3
rs2814944_G	0.49	6/C6orf106	21.7	rs1042034_T	5.99	2/APOB	74.3	rs3757354_C	1.43	6/MYLIP	75.1
rs4731702_A	0.59	7/KLF14	54.4	rs10761731_A	2.38	10/JMJD1C	63.1	rs1800562_G	2.22	6/HFE	96.5
rs2923084_A	0.41	11/AMPD3	13.6	rs11613352_C	2.70	12/LRP1	77.3	rs1564348_T	0.56	6/LPA	14.3
rs7134375_A	0.40	12/PDE3A	59.2	rs11649653_C	2.13	16/CTF1	61.3	rs11220462_A	1.95	11/ST3GAL4	18.2
rs7134594_A	0.44	12/MVK	53.2	rs11776767_C	2.01	8/PINX1	30.2	rs8017377_A	1.14	14/NYNRIN	37.2
rs1532085_A	1.45	15/LIPC	42.4	rs1260326_A	8.76	2/APOB	35.4	rs6511720_G	6.99	19/LDLR	10.3
rs3764261_A	3.39	16/CETP	73.6	rs12678919_A	13.64	8/LPL	91.2	rs2479409_G	2.01	1/PCSK9	28.6
rs2925979_C	0.45	16/CMIP	31.7	rs13238203_C	7.91	7/TYW1B	97.2	rs629301_T	5.65	1/SORT1	78.1
rs4148008_C	0.42	17/ABCA8	31.4	rs1495741_G	2.85	8/NAT2	75.1	rs1367117_A	4.05	2/APOB	30.5
rs4129767_A	0.39	17/PGS1	36.5	rs17145738_C	9.32	7/MLXIPL	88.0	rs11136341_G	1.40	8/PLEC1	36.2
rs737337_A	0.64	19/LOC55908	8.0	rs174546_A	3.82	11/FADS1-2-3	40.3	rs7206971_A	0.78	17/OSBPL7	48.1
rs1800961_C	1.88	20/HNF4A	4.3	rs2068888_G	2.28	10/CYP26A1	54.1	rs4420638_G	7.14	19/APOE	25.1
rs6065906_T	0.93	20/PLTP	14.6	rs2131925_T	4.94	1/ANGPTL3	72.6	rs6029526_A	1.39	20/TOP1	53.3
rs1689800_A	0.47	1/ZNF648	26.9	rs2247056_C	2.99	6/HLA	76.2				
rs4846914_A	0.61	1/GALNT2	46.1	rs2412710_A	7.00	15/CAPN3	3.1				
rs12328675_C	0.68	2/COBLL1	9.8	rs2929282_T	5.13	15/FRMD5	6.2				
rs2972146_G	0.46	2/IRS1	36.4	rs2954029_A	5.64	8/TRIB1	54.3				
rs6450176_G	0.49	5/ARL15	14.6	rs439401_C	5.50	19/APOE	72.4				
rs605066_T	0.39	6/CITED2	47.9	rs442177_T	2.25	4/KLH8	52.4				
rs1084651_A	1.95	6/LPA	17.4	rs5756931_T	1.54	22/PL2G6	60.3				
rs9987289_G	1.21	8/PPP1R3B	15,5	rs645040_T	2.22	3/MSL2L1	85.8				
rs2293889_G	0.44	8/TRPS1	70.9	rs9686661_T	2.57	5/MAP3K1	14.3				
rs581080_C	0.65	9/TTC39B	87	rs964184_C	16.95	11/APOA1	14.1				
rs1883025_C	0.94	9/ABCA1	80.1								
rs3136441_C	0.78	11/LRP4	22.7								
rs4759375_T	0.86	12/SBNO1	9.2								
rs4765127_T	0.44	12/ZNF664	28.4								
rs838880_C	0.61	12/SCARB1	41.9								
rs2652834_G	0.39	15/LACTB	76.3								
rs16942887_A	1.27	16/LCAT	15.9								
rs11869286_C	0.48	17/STARD3	32.1								
rs7241918_T	1.31	18/LIPG	83.6								
rs12967135_G	0.42	18/MC4R	82.4								
rs7255436_A	0.45	19/ANGPTL4	51.5								
rs386000_C	0.83	19/LILRA3	29.7								
rs181362_C	0.46	22/UBE2L3	67								
rs13107325_G	0.84	4/SLC39A8	98.7								

Note: The gene name listed under 'locus' is either plausible biological candidate gene in the locus or the nearest annotated gene to the SNP. 'chr' denotes chromosome. 'Freq (%)' denotes the risk allele frequency in the YF population, Adapted from Teslovitch et al. (2010)[258] and Tikkanen et al. (2011) [267].

Appendix 3.6. Child and adult cutoffs used to define 'normal' and 'high-risk' serum lipid levels (in mmol/L).

Childhood cutoffs apply to participants 19 years and younger. Chosen cutoffs are based on NCEP adult-panel treatment guidelines, NCEP pediatric and adolescent guidelines and AAP and AHA pediatric guidelines (see methods).

		Normal Risk	High Risk
HDL-C	Childhoood	> 0.91	≤ 0.91
	Adulthood	> 1.03	≤ 1.03
LDL-C	Childhoood	< 4.14	≥ 4.14
	Adulthood	< 3.37	≤ 3.37
Triglycerides	Childhoood	<1.4	≤ 1.4
	Adulthood	< 1.7	≤ 1.7

Appendix 3.7. Sex-specific associations between categorical genetic risk score and lipid levels at selected ages (3 years, 15 years, 24 years and 45 or 46 years).

Effect sizes β are in mmol/L for the number of risk allele differences between high and low wGRSs. Reported at each age are: Int (SE), the average lipid level in mmol/L (SE) in the high risk score group; β (SE), the difference in average lipid level in mmol/L for subjects in the low risk score group, the p-value for each cross-sectional association and the number of non-missing serum lipid observations considered in each regression (Nobs). (*: For each sex, the number of risk alleles difference between high and low wGRS groups are calculated as*: median (Number of alleles in the High wGRS) – median ((Number of alleles in the Low wGRS))*.

		wGRS	Number of risk alleles	Average Lipid Levels			
				3 years	15 years	24 years	45/46 years
LDL-C	*Males*	High	16.72	Int: 3.57 (0.13)	Int: 3.01 (0.11)	Int: 3.23 (0.13)	Int: 3.68(0.14)
		Low	12.19	β: -0.49 (0.12)	β: -0.42(0.12)	β: -0.45 (0.11)	β: -0.56 (0.10)
				P-val=0.005	P-val=$9.1*10^{-7}$	P-val=$8.3*10^{-6}$	P-val=$9.7*10^{-8}$
				Nobs=173	Nobs=617	Nobs=313	Nobs=421
	Females	High	16.68	Int: 3.78 (0.11)	Int: 3.31 (0.10)	Int: 3.16 (0.10)	Int: 3.57 (0.11)
		Low	12.55	β:-0.35 (0.11)	β: -0.39 (0.09)	β: -0.41 (0.11)	β: -0.51 (0.10)
				p-val=0.023	P-val=$3.8*10^{-6}$	P-val=$2.1*10^{-5}$	P-val=$2.0*10^{-8}$
				Nobs=184	Nobs=711	Nobs=397	Nobs=475
HDL-C	*Males*	High	48.08	Int: 1.56 (0.08)	Int: 1.48 (0.06)	Int: 1.3 (0.08)	Int: 1.40 (0.07)
		Low	40.99	β: -0.15 (0.04)	β: -0.15 (0.05)	β: -0.13 (0.03)	β: -0.16 (0.07)
				P-val=0.025	P-val=$5.8*10^{-6}$	P-val=0.01	P-val=$1.3*10^{-4}$
				Nobs=161	Nobs=594	Nobs=293	Nobs=415
	Females	High	48.00	Int: 1.56 (0.06)	Int: 1.62 (0.06)	Int: 1.56 (0.04)	Int: 3.01 (0.08)
		Low	41.01	β: -0.16 (0.05)	β: -0.15 (0.04)	β: -0.13 (0.04)	β: -0.17 (0.05)
				P-val=0.004	P-val=$2.5*10^{-6}$	P-val=0.01^{7}	P-val=$3.7*10^{-5}$

				Nobs=174	Nobs=688	Nobs=373	Nobs=446
Triglycerides	*Males*	High	28.99	Int: 0.70 (0.08)	Int: 0.96 (0.1)	Int: 1.5 (0.1)	Int: 1.77 (0.15)
				β: -0.17 (0.05)	β: -0.23 (0.03)	β: -0.46 (0.03)	β: -0.53 (0.04)
		Low	23.02	P-val=0.001	P-val=$4.3*10^{-8}$	P-val=$5.8*10^{-5}$	P-val=$3.5*10^{-6}$
				Nobs=173	Nobs=620	Nobs=315	Nobs=439
	Females	High	28.99	Int: 0.75 (0.09)	Int: 0.93(0.08)	Int: 1.33 (0.07)	Int: 1.65 (0.08)
				β: -0.19 (0.07)	β: -0.17(0.05)	β: -0.20 (0.06)	β: -0.23 (0.07)
		Low	23.07	P-val=$8.5*10^{-6}$	P-val=$1.1*10^{-6}$	P-val=0.0001	P-val=$1.5*10^{-8}$
				Nobs=186	Nobs=717	Nobs=393	Nobs=470

Appendix 3.8. Step by step IGC model-building procedure, explicit parametrisation of the IGC submodels and references on IGC modeling.

Data exploration

Prior to all IGC analyses, plotting raw data trajectories of a number of randomly selected YFS participants suggested variability in individuals' initial lipid levels and variability in how individuals' lipid levels change as they age. The shape of each lipid trajectories across the life course was examined using individual empirical growth plots and generalised additive mixed models (GAMMs). GAMM is a well-suited modeling framework for uncovering potentially non-linear trends in correlated datasets. This approach allows flexible specification of regression splines to represent the functional relationships between a response variable and a temporal covariate that can be continuous or discrete (i.e. age) using smooth functions of the covariate ('age' in this case). The fitting of GAMMS can also suggest plausible polynomial models to describe the pattern of change in the lipid model (i.e. typically the model order is chosen by the number of bends needed in the predicted GAM trajectory (number of inflection points)).

Note: To conduct IGC analysis, three or more waves of data collection are required; having two waves or less will limit the ability to identify shape of individual growth trajectories and to distinguish true growth from measurement error. Theoretically, it is possible to test for linear growth with a minimum of three data waves per subject, quadratic growth with four waves minimum, and so one. In this study sample there is a maximum of eight waves of data for each YFS participant so an age polynomial up to the order seven could be fit in theory. However, in practice, because participants had 5.31 lipid observations on average, the highest order age polynomial term considered to describe individual lipid profiles was age^4 (i.e. quartic age growth).

Individual Growth Curve modeling (IGC analysis)

IGC modeling is a multilevel regression technique used for exploring longitudinal data on individuals over time. It allows investigating two levels of variability of the response variable: within and between subjects. In longitudinal data, measurements made on the same individual are correlated and it is this dependency that leads to the inadequacy of simple estimation procedures based on ordinary least squares. Observations taken over time are nested within subjects drawn from a population of interest giving a two-level hierarchical

structure. The variation of responses within subjects over time is at the lowest level (level-1) and the variation of the underlying mean responses between subjects is at level-2 [23]. Growth curve analysis allows to model and quantify change over time both at group-level (level-2) and individual-level (level 1). Sometimes in longitudinal data, the interest lies as much in the covariance matrix estimates as in the average growth parameters. IGC allows examination of both. In this case, the time-relevant predictor is age and the predictor of interest is genetic risk scores (wGRSs) as the purpose was to determine if wGRSs modify the trajectories of blood lipids across the lifetime. Because males and females have been shown to differ in their lipid trajectories profiles across the life course in previous research, a test of how 'sex' modifies the lipid trajectories was conducted. In practice, IGC modeling consists of sequentially testing a number of models as follows:

Step 1) Unconditional means (UM) model

The UM model is the simplest multilevel model containing no predictor (i.e. this is often viewed as a one-way ANOVA model with subject-level random intercept). It serves as a baseline model to examine individual variation in the outcome variable without regard to age. This model assesses (1) the grand mean of the outcome variable, and (2) the amount of outcome variation that exists at the intra- and inter-individual levels. It allows computing the ICC (intraclass correlation coefficient), which describes the amount of variance in the outcome that is attributed to differences between individuals. The ICC evaluates the necessity of modeling the nested data structure. For LDL-C, the UM model can be written as:

Level 1: $LDL_{ij} = b_{oi} + e_{ij}$

Level 2 : $b_{oi=} \beta_0 + u_{0i}$

Where $e_{ij} \sim_{iid} N(0, \sigma^2)$ and $u_{0i} \sim_{iid} N(0, \tau^2)$

Example of UM model for LDL-C in R notation (`nlme` package):

```
UMmodel<- lme (LDL-C ~1, random= 1|subject,  method = "ML",
na.action = na.omit)
```

For example, when applying the UM model to LDL-C, an ICC of 0.67 was calculated, suggesting that about 67% of the total variation in the LDL-C level was due to inter-individual difference, strongly supporting a multilevel model approach.

Step 2) Potential unconditional growth (UG) models

The next step is to determine the Unconditional growth model (UG model), which in this case is a model where the response variable (i.e. lipid level) is modeled as a function of age, and where participant IDs are used as a random effect (i.e. random intercept). This is the level-1 model, which describes how each individual lipid levels change over time. In UG models, subjects are collapsed across all possible grouping variables of interests (i.e. sex, risk factors, etc), and age (or any other relevant time variable) is expressed as a deviation from average value in the cohort (i.e. centering the time-related variable will reduce multicollinearity between polynomial terms if a higher order polynomial is needed to fit the data).

It is common practice to always fit an Unconditional Linear UG Model, even when data exploration suggests a non-linear response over time. The linear UG model is used as a baseline to compare curvilinear UG models for the response over time. Orthogonal polynomials are a possible choice to model non-linear response variables by IGC analysis. Unlike natural polynomials, orthogonal polynomials terms have the advantage to be interdependent (no multicollinearity between age, age^2, age^3…). When using orthogonal polynomials, the intercept term of the model will reflect the average overall curve height, rather than the height at the left edge of the time window, but higher order parameters are difficult to interpret. They are usually preferred when the main interest lies in prediction.

For this application, natural polynomial terms were used to capture the curvature of the lipid profiles to determine if differences existed at the very beginning of the time window (i.e. age was centered around the youngest age in the cohort (age 3)). Also, the pattern of change in the response over time rather than which time component, was more important. Therefore, it was more desirable to interpret the output of the linear mixed effect models on the original time-scales. Additionally, collinearity between covariates does not tend to affect the quality of parameter estimation to a notable extent, so the age terms were centered (at around 3 years old) rather than using orthogonal polynomials as recommended by Liu and Engel 2015[86]

Example : Potential UG models for LDL-C:

 # linear UG model (with a random intercept only):

Level 1: $LDL_{ij} = b_{oi} + b_1.Age_{ij} + e_{ij}$ # Addition of age as a predictor at level 1

Level 2 : $b_{oi=}\beta_0 + u_{0i}$

Where $e_{ij} \sim_{iid} N(0, \sigma^2)$ and $u_{0i} \sim_{iid} N(0, \tau^2)$

In lme syntax, the linear UG can be written as:

```
Linear. UG<- lme (LDL-C ~ age, random= 1|subject, method =
"ML", correlation, na.action = na.omit)
```

However, the fitting of GAM models in the data exploration step suggested that the lipid responses were curvilinear, modeled with a cubic or 4th order polynomial polynomial. Therefore, the following potential UG models were fitted:

 # Quadratic UG model (with a random intercept):

Level 1: $LDL_{ij} = b_{oi} + b_1.Age_{ij} + b_2.Age^2_{ij} + e_{ij}$ # Addition of age-square as a predictor

Level 2 : $b_{oi=}\beta_0 + u_{0i}$

Where $e_{ij} \sim_{iid} N(0, \sigma^2)$ and $u_{0i} \sim_{iid} N(0, \tau^2)$ with e_{ij} and u_{0i} are independent for all *i* and *j*.

In R notation, this model is written:

```
Quadratic. UG<- lme (LDL-C ~ age+ I(age^2)+ , random=
1|subject,    method = "ML", correlation, na.action = na.omit)
```

Similarly, more complex level-1 submodels can be defined by including cubic or quartic growth predictors:

 # Cubic UG model (with a random intercept)

```
cubic. UG<- lme (LDL-C ~ age+ I(age^2)+ I(age^3) , random=
1|subject,    method = "ML", correlation, na.action = na.omit)
```

 # Quartic UG model (with a random intercept)

```
quartic. UG<- lme (LDL-C ~ age+ I(age^2)+ I(age^3)+ I(age^4)+
, random= 1|subject,    method = "ML", correlation, na.action
= na.omit)
```

Unconditional Growth Model Comparisons:

When comparing increasingly complex submodels throughout the IGC analyses, the improvement in model fit is assessed by the Likelihood Ratio Test (LRT) or by looking at AIC/BIC criteria. The best fitting UG model among these candidate level-1 submodelswas determined using both criteria.

1*) likelihood ratio tests (LRT):* can only be used to compare models nested within one another (i.e. this is done by computing -2 times the difference between the two models residual log likelihoods (-2RLL), and comparing it to the χ^2 distribution with degrees of freedom equal to the difference in the number of parameters for the two models). Models are preferred where the -2RLL is smaller.

2) *Akaike's Information Criterion (AIC)* is also useful as it is valid to compare non-nested models (i.e. the model with the smallest AIC is preferred).

It is important that the candidate level 1 models are fit using full maximum likelihood (method="ML" argument in R), so that the LR tests can be used to evaluate the elimination of either fixed or random effects. The adequate UG model will then be retained as the baseline model for the next steps in the IGC analysis.

Example:

```
anova(UMmodel ,linear.UG, quadratic.UG, cubic.UG, quartic.UG)
AIC(UMmodel ,linear.UG, quadratic.UG, cubic.UG, quartic.UG)
```

At this stage, AIC criteria and LR tests showed the cubic growth model with variance in individual intercept was best at capturing the intra-individual growth of LDL-C over time.

So far, LDL-C UG model, with age_{ij} centered around 3 years old, can be written:

Level 1 : $Y_{ij}=b_{oi}+b_1(age_{ij}) + b_2(age_{ij})^2 + b_3(age_{ij})^3 + e_{ij}$

Level 2 *:* $b_{oi=}\beta_0 + u_{0i}$

With e_{ij},the amount the response on occasion j deviates from person i's true change trajectory.

Step 3) Model the random effect structure (i.e. subject-level intercept and slope variability)

Once the best UG model has been determined, it is possible to explicitly model the random part of this model. Indeed, a potential limitation of the UG model chosen above for LDL-C (i.e. cubic growth model with random intercept) is that is assumes the relationship between time and LDL-C is constant for all individuals. An alternative model is one where unobserved individual characteristics would also explain variation in trajectory parameters. This means expanding the level 2 model by adding random terms for age-, age^{2-}, and age^{3} slopes.

There are diverging views in the literature regarding the specific modeling of random effects in IGC analysis. Barr et al. [375], do not advocate model selection of the random growth parameters, suggesting that UG models should include all random effects that are licensed by the design (i.e. all the ones that could potentially vary across participants). This is sometimes referred to as the 'beyond optimal random effect structure', where all age terms in the fixed effect part of the growth model also appear in the random part of the model. The reason for allowing all possible slopes to vary across individual is because when a random effect does not improve model fit, it can still affect the fixed effect estimates and excluding it can elevate the false positive rate. However, estimating random effects is "expensive" in terms of the number of observations required, and estimating too many random effects can lead to computational difficulty (i.e. model failing to converge). Mirman et al.[18, 19] include participant random effects on all time terms up to the cubic, as terms beyond this tends to capture less-relevant effects in the tails. Others, including Pinheiro et al. [114], recommend testing the significance of all possible random terms when higher-level trends are significant, to determine if allowing the slopes of higher-level variables to vary randomly will improve model fit (i.e. test that a quadratic or cubic relationship varies in strength among individuals).

The approach used here, when technically possible, was to test sequentially if each additional random parameter (i.e. random intercept, random linear slope, random quadratic slope…) improved the fit of the unconditional UG models using LR tests and AIC, as in Zuur et al. and Bliese [121, 376]. This is done in our model by adding, in order of increasing complexity, the linear, quadratic, and cubic age terms as random effects in the cubic UG growth model. If one

wants to select at once of the optimal fixed effect and random effect structure, it is best to use ML estimation rather than REML [114]. Here, because it was determined that the best model was the cubic UG mode, the restricted maximum likelihood "REML" estimation method was used, so that LRT tests evaluate the elimination of random effects only.

Once the optimal random effect structure has been chosen for the UG model, one can visualise the variance covariance matrix of the random effects at level 1 (i.e for lme objects in R, one can access random effect variance and correlation estimates using the VarCorr () argument). It will reveal, for example, if within-individual lipid levels at baseline (intercept) are correlated with linear change in lipid across the life course. As is common with random effects, we apply no constraint on the variance-covariances of the random effect matrix (i.e. unstructured G-matrix).

Example: with age centered around 3 years old, LDL-C UG sub-models with slope variability include:

 # cubic UG model (with a random intercept) == best level 1 model as determined by LR test above

```
cubic. UG<- lme (LDL-C ~ age+ I(age^2)+ I(age^3) , random=
1|subject,    method = "REML", correlation, na.action =
na.omit)
```

 # cubic UG model with a random intercept and linear age slope: (2X2 unstructured variance covariance matrix): At this point we have three variance components: σ^2, τ_0^2 and τ_1^2 . The correlation between intercept and linear slope is given by τ_{01}.

Level 1 : $Y_{ij}=b_{oi}+b_{1i}(age_{ij}) + b_2(age_{ij})^2 + b_3(age_{ij})^3 + e_{ij}$

Level 2 : $b_{oi}=\beta_0+u_{0i}$

$b_{1i}=\beta_1+u_{1i}$

Where in addition : $e_{ij} \sim_{iid} N(0, \sigma^2)$ and $u_i=\begin{bmatrix}u_{oi}\\u_{1i}\end{bmatrix} \sim_{iid} N\left(\begin{bmatrix}o\\o\end{bmatrix}, \begin{bmatrix}\tau_o^2 & \tau_{01}\\\tau_{01} & \tau_1^2\end{bmatrix}\right)$

In R notation:

```
cubic. UG. 1<- lme (LDL-C ~ age+ I(age^2)+ I(age^3) , random=
1+age|subject,  method = "REML", correlation, na.action =
na.omit)
```

It is also possible to add additional variance components at level 2 (i.e. random quadratic, and cubic slopes):

 # cubic UG with a random intercept and random linear and quadratic slopes: (3X3 unstructured variance covariance matrix)

Level 1 : $Y_{ij}=b_{oi}+b_{1i}(age_{ij}) + b_{2i}(age_{ij})^2 + b_3 (age_{ij})^3 + e_{ij}$

Level 2 : $b_{oi}=\beta_0+u_{0i}$

$b_{1i}=\beta_1+u_{1i}$

$b_{2i}=\beta_2+u_{2i}$

Where in addition : $e_{ij} \sim_{iid} N(0, \sigma^2)$, and $u_i=\begin{bmatrix}u_{0i}\\u_{1i}\\u_{2i}\end{bmatrix} \sim \text{iid}\left(\begin{bmatrix}o\\o\\o\end{bmatrix}, \begin{bmatrix}\tau_O^2 & \tau_{01} & \tau_{02}\\ \tau_{01} & \tau_1^2 & \tau_{12}\\ \tau_{02} & \tau_{12} & \tau_2^2\end{bmatrix}\right)$

In R notation, this can be written as:

```
cubic. UG. 2<- lme (LDL-C ~ age+ I(age^2)+ I(age^3) , random=
1+age+ I(age^2)|subject,    method = "REML", correlation,
na.action = na.omit)
```

For a level 1 model with a 3rd order polynomial, the maximum possible random parameters for the variance covariance matrix is 4x4:

 # cubic UG with a random intercept and random linear, quadratic and cubic slopes:

```
cubic. UG. 3<- lme (LDL-C ~ age+ I(age^2)+ I(age^3) , random=
1+age+ I(age^2) + I(age^3)|subject,    method = "REML",
correlation, na.action = na.omit)
```

To determine the best random effect structure, the increasingly complex submodels (in terms of additional variance and covariance components for the random effects) are then compared using AIC or LR tests.

mod.aic<-sapply(list(cubic. UG, cubic. UG. 1, cubic. UG. 2, cubic. UG. 3), AIC)
LR-test<-sapply(list(cubic. UG, cubic. UG. 1, cubic. UG. 2, cubic. UG. 3), anova)

Example:
In the case of the LDL-C UG model, all 4-candidate structures for random effects could be estimated with no convergence issue. The cubic growth model with a random intercept, linear and quadratic slopes was the best fit to the data as determined by LR test and AIC criteria (with a 3 x 3 unstructured variance covariance matrix for the random effects). In this model. τ_0^2 *refer to the variability in the intercepts of the subject-specific regression lines about the intercept of population-averaged line,* τ_1^2 *is the variability of the individual slopes about the population linear slope, and* τ_2^2*, the variability of the individual slopes about the population line quadratic slope.* σ^2 *is the average variability of participants LDL-C values about their own individual regression line.*

Step 4) Model residual error structure

It is very important to scrutinize level-1 error structure especially with unequally spaced and unbalanced data, because estimated variances of the parameter estimates are likely to be biased and affect the precision of estimating the appropriate model. The purpose of testing different error covariance matrices is to describe how UG model the error is distributed. It is likely that lipid measurements taken temporally close to each other will be more strongly related than those temporally far apart (i.e. autocorrelation). It is also likely that lipid levels will tend to become either more variable over time or less variable over time as participants age (i.e. heteroscedasticity). UG models need to be tested for both autocorrelation and heteroscedasticity. The Models fitted to select the optimal error structure are also fitted using ML (maximum likelihood) [121].

A/ Residual correlation structure:

In the present study, three commonly examined types of error covariance structures were tested for the UG models: unstructured, compound symmetric, and first-order autoregressive variance covariance of the residuals' matrix (Pinheiro and bates 2013[114]):

1) *Unstructured (UN)*, often provides the best fit in longitudinal data as it requires no assumption in the error structure. The variance is not assumed constant between different ages and the correlations between observation times can all be different (estimation can be problematic as many parameters as number of timepoints -1 need to be estimated). In lme notation such a residual correlation structure is defined as: `correlation = corSymm(form=~1|subject)` within the model formulation.

2) *Compound Symmetry (CS)*, implies that the variance and correlation between each pair of observations are constant across time points (i.e. `correlation = corCompSymm (form=~1|subject)`)

3) *Continuous First-Order Autoregressive (AR1)*, in which the variance is assumed to be heterogeneous and the correlations between the two adjacent time points decline across measurement occasions (i.e. `correlation = corCAR1(form = ~ age| subject).`

Example:
In the LDL-C UG model example, the LR-test showed that a model with an AR1 error structured fit the data better, so it will be retained when introducing level 2-predictors in the model.

B/ Residual heteroscedasticity:
Examining the variance of lipid concentrations at each age can give an idea of variance homogeneity. For each lipid UG model the error variance decrease or increase over ages was tested (i.e. `weights=VarFixed(form=~age)` or `weights=VarExp(form=~age)` arguments in the lme() call respectively).

Example: For all lipid UG models, residual variance did not exhibit significant increasing or decreasing trends over time so that subsequent models were not adjusted for heteroscedasticity.

Step 5) The conditional growth (CG) sub-models

Once the fixed and random components of the unconditional growth model have been determined, the independent predictors (time-varying or time invariant) can be introduced into the lipid growth model: it becomes the conditional growth model. This level-2 model (i.e. between-person model) describes how the individual growth differs across individuals. The CG model represents the relationship between level 1 growth parameters (intercepts, linear, quadratic and cubic slopes) and time-invariant characteristics of the individuals (e.g. in this application, wGRS group ('low', 'high', "mid') or continuous wGRS; and sex). The Level-2 model captures whether the rate of change varies across individuals in a systematic way. The growth parameters (i.e. the within-subject intercepts and slopes) of Level-1 are the outcome variables to be predicted by the between-subjects variables at Level 2. The CG model allows questions such as "are there differences between genetic risk group (High vs low) in the lipid change over time?", or " How does a 1-SD increase in wGRS will impact the average lipid trajectory as a person age?" to be answered.

1- <u>Add sex as level-2 predictor in the model:</u>

```
table(LDLgrsdata$sex)

Female   Male
  5192   4544
```

Sex is a factor variable in R. By default dummy variables have been constructed to contrast temperate and male with female participants:

```
contrasts(LDLgrsdata$sex)
       Male
Female    0
Male      1
```

Now it is possible to explore how "sex' modifies the basic level-1 relationship between LDL-C and age. It can potentially affect the intercept, the slopes, or both. Similar to what is described above, we can sequentially test the 3 following models:

1- Does sex affect the population LDL-C intercept (LDL-C levels at age 3) ?

Baseline level 1 model for comparison:

```
cubic. UG. 2<- lme (LDL-C ~ age+ I(age^2)+ I(age^3) , random=
1+age+ I(age^2)|subject,    method = "ML", correlation,
na.action = na.omit)

model3<-lme(LDL-C~I(LDL-C ~ age+ I(age^2)+ I(age^3) +sex,
random= 1+age+ I(age^2) |subject,    method = "ML",
correlation, na.action = na.omit)

anova(model3, cubic. UG. 2)# LR-test, P-val= 0.45
```

Note: because 'sex' is a categorical variable with only two categories, the Wald tests shown in the summary output of model 3 will provide a test for the significance of "sex" for the intercept (this will not be different from the LR-test). However, it is good practice to use Likelihood Ratio testing, as for variables with more than 2 levels, it is necessary to apply LR-test to obtain significance of the variable.

It is possible to further quantify the relationship between sex and the intercept of the LDL-C model by computing a pseudo-R^2 statistic:

$$\text{Level 2 Pseudo-}R^2 = \frac{\tau^2(\text{unconditional growth model}) - \tau^2(\text{subsequent model})}{\tau^2(\text{unconditional growth model})}$$

```
as.numeric(VarCorr(cubic. UG. 2)[2,1]) -
as.numeric(VarCorr(model3)[2,1])) / as.numeric(VarCorr(cubic.
```

```
UG. 2)[2,1])= 0.04
```

So only 3% of the individual-level variation in the intercept (i.e. LDL-C levels at age 3) is explained by "sex". This is consistent with the fact that adding "sex" to the level-2 equation for the intercept did not significantly improve the model (LR test, p-value=0.45)

2- Does sex affect the population linear slope?

Similarly to what has been shown above, we can compute:

```
model4<-lme(LDL-C~I(lntemp-~ age+ I(age^2)+ I(age^3) +sex:age,
random= 1+age+ I(age^2) |subject, method = "ML", correlation,
na.action = na.omit)

anova(model4, cubic. UG. 2)
```

The likelihood ratio test finds the effect of climate on the slope to be statistically significant. This is confirmed by looking at the AIC. We can also quantify the effect with a pseudo-R^2 statistic (i.e. this time it compares the change in τ_1^2 between the current model and random slopes and intercepts model (cubic.UG.2 model)):

```
(as.numeric(VarCorr(cubic. UG. 2)[2,1]) -
as.numeric(VarCorr(model3)[2,1])) / as.numeric(VarCorr(cubic.
UG. 2)[2,1])= 0.04
```

3- Does sex affects the all population trajectory parameters?

Following the same approach, we use LR-T test, AIC and level 2 pseudo-R^2 to test sequentially the effect of sex on every trajectory parameter:

The most complex model, where sex affects all trajectory parameters, can be written as:

Level 1 : $Y_{ij}=b_{oi}+b_{1i}(age_{ij}) + b_{2i}(age_{ij})^2 + b_3(age_{ij})^3 + e_{ij}$

Level 2 : $b_{oi}=\beta_0+\beta_{01}(sex_i)+u_{0i}$

$b_{1i}=\beta_1+\beta_{11}(sex_i)+u_{1i}$

$b_{2i} = \beta_2 + \beta_{21}(sex_i) + u_{2i}$

In R notation:

```
model5<-lme(LDL-C~I(lntemp-~ (age+ I(age^2)+ I(age^3) )*sex,
random= 1+age+ I(age^2) |subject,    method = "ML",
correlation, na.action = na.omit
anova(model5, cubic. UG. 2)
```

AIC and LR tests showed that model 5 was the best level 2 model for LDL-C at this stage.

2- <u>Add wGRS risk group as level-2 predictor in the model:</u>

Similar to what was demonstrated above for 'sex' above, the influence of wGRS group on individual growth parameters of LDL-C can be tested. In this mode,l contrasts where chosen to compare low and mid genetic risk groups with high genetic risk category:

```
contrasts(LDLgrsdata$GRS)
     low mid
high   0   0
low    1   0
mid    0   1
```

Similar to what was demonstrated for sex, the effect of GRS groups on LDL-C intercept and age slopes are tested using LR test and AIC.

Example: Hypothetic level-2 model for LDL-C with wGRS group affecting all trajectory parameters:

Level 1 : $Y_{ij} = b_{oi} + b_{1i}(age_{ij}) + b_{2i}(age_{ij})^2 + b_3(age_{ij})^3 + e_{ij}$

Level 2:

$b_{oi} = \beta_{00} + \beta_{02}(wGRS_i) + \zeta_{0i}$ # individual intercept

$b_{1i} = \beta_{10} + \beta_{12}(wGRS_i) + \zeta_{1i}$ # individual linear slope

$b_{2i} = \beta_{20} + \beta_{22}\ (wGRS_i) + \zeta_{2i}$ # individual quadratic slope

$b_3 = \beta_{30} + \beta_{31}\ (wGRS_i)$

3- ***Test the combined effects of sex and wGRSs groups and their interaction as level 2 predictors: Final IGC model***

The final IGC model is the one where all level-2 predictors and their cross-products are added in the model. It allows the effect of the dichotomous genetic risk score category, sex, and their interaction (wGRS group by sex) on the shape of average LDL-C growth trajectories in the cohort to be tested. It is used to explore any group differences in change over time. The corresponding final IGC model can be written as:

```
LDLserum.lme <- lme(LDLserum ~ sex*(age+I(age^2)+I(age^3))+
LDLwGRS14*(age+I(age^2)+I(age^3)) + (sex* LDLwGRS14)* (age+I(age^2)+I(age^3)) ,
random=list(Patients=~1+age+ I(age^2)),
                method = "ML",correlation = corCAR1(form = ~ age| subject),data =
LDLgrsdata,na.action = na.omit,control=lmeControl(opt="optim"))
```

Final IGC model, with level-2 predictors sex and wGRSs groups and their crossproducts:

Level 1 : $Y_{ij} = b_{oi} + b_{1i}(age_{ij}) + b_{2i}(age_{ij})^2 + b_3\ (age_{ij})^3 + e_{ij}$

Level 2:

$b_{oi} = \beta_{00} + \beta_{01}\ (sex_i) + \beta_{02}\ (wGRS_i) + \beta_{03}\ (sex_i * wGRS_i) + u_{0i}$ # individual intercept

$b_{1i} = \beta_{10} + \beta_{11}\ (sex_i) + \beta_{12}\ (wGRS_i) + \beta_{13}\ (sex_i * wGRS_i) + u_{1i}$ # individual linear slope

$b_{2i} = \beta_{20} + \beta_{21}\ (sex_i) + \beta_{22}\ (wGRS_i) + \beta_{23}\ (sex_i * wGRS_i) + u_{2i}$ # individual quadratic slope

$b_3 = \beta_{30} + \beta_{31}\ (sex_i) + \beta_{32}\ (wGRS_i) + \beta_{33}\ (sex_i * wGRS_i)$ # population average cubic slope

with :

- β_{00}, population level average intercept (fixed effect) and β_{10} = population average linear slope, $\beta_{20=}$=population average quadratic slope, b3=population average quadratic slope.
- β_{01}, β_{02}, and β_{03}: three predictors of intercept variability, respectively for sex, wGRS and sex*wGRS interaction.
- u_{0i}, u_{1i}, u_{2i}, the amount person i's intercept, linear slope, and quadratic slopes deviate from the population average parameters.
- e_{ij}. the amount the response on occasion j deviates from person i's true change trajectory, and e_{ij} follows a AR1 structure.

The significance of each predictor in the final UG model is assessed by t-statistics for the parameter *(i.e. defined as the ratio of parameter estimate and SE).* Reported in the Tables 3.2 and 3.3 are the p-values provided in the LME () output in R.

Example: Parameter estimates presented in Table 3.2 and 3.3 for LDL-C correspond to β_{02}, β_{12} β_{22} and β_{32} parameters of the final IGC models, as all sex wGRS interaction terms (i.e. β_{03}, β_{13}, β_{23} and β33) were unsignificant in the final model. The fixed estimates from the final IGC model output can be used to compute the slope estimates for the 6 relevant groups (i.e. male –High risk, male-mid risk, male -low risk, female-high risk, female-mid risk and female low-risk) (Figure 1).*

IGC modeling vs. ANOVA?

The specification of an IGC model is different to an ANOVA model: Although individual growth curve models can treat age as a continuous variable across subjects, and model change in outcome at the individual level, the ANOVA model focuses on growth curves at the aggregate level. ANOVA assumes that all individuals are measured at the same time-point for any given wave of data collection. One of the advantages of IGC models over ANOVA, is that they can treat age as a truly continuous variable. ANOVA also does not model individual variability in rates of change in the outcome over time.

Adjustment for confounding effects:

1- Birth cohort effect

To minimise potential confounding effects, a birth cohort effect on the age-related changes in lipids in the YF cohort was considered for each sex. Before introducing genetic risk scores (wGRSs) as predictors of lipid trajectories, the effect of 'year of birth' (yob) on each lipid's unconditional growth model was thus assessed. This was performed by adding the variable "yob" and its interaction terms with age-related terms to each sex-specific lipid trajectory model (i.e. for female LDL-C model, for example: age, age^2, age^3, yob, yob*age, yob*age^2 and yob*age^3). "yob" was used as a categorical variable with 6 levels, for the 6 possible birth cohorts represented in the data sample (i.e. participants born in 1977, 1974, 1971, 1968, 1965, and 1962 respectively). As described above for sex and wGRSs group, LRT test and AIC were used to test the significance of a birthcohort effect on trajectory parameters for each lipid.

2-Period effect

Before considering the predictive effect of the genetic risk scores on the lipid trajectories, the potential differences in lipid measurements between examinations or secular 'period effect' in lipids over the 31 years of follow-up were examined. To test for this, calendar 'year' (centered around 1980 (baseline)) was introduced in the sex-specific lipid unconditional growth models (i.e. for LDL-C the model included age, age^2, age^3, year, year*age, year*age^2 and year*age^3).

Example:

In all sex-specific analyses of lipid profiles, we found no birth cohort effect. However, in each case, we found significant secular trends (see 3.4.2.). For this reason, all UG models were adjusted for 'year' before introducing subject-level trajectory predictors (i.e. wGRSs). For example, the final IGC model for LDL-C can be written as:

LDLserumlme <- lme(LDLserum ~ sex(age+I(age^2)+I(age^3)))+*
LDLwGRS14(age+I(age^2)+I(age^3))) + (sex* LDLwGRS14)* (age+I(age^2)+I(age^3)))*
*+**as.numeric(year),** random=list(Patients=~1+age+ I(age^2)),*

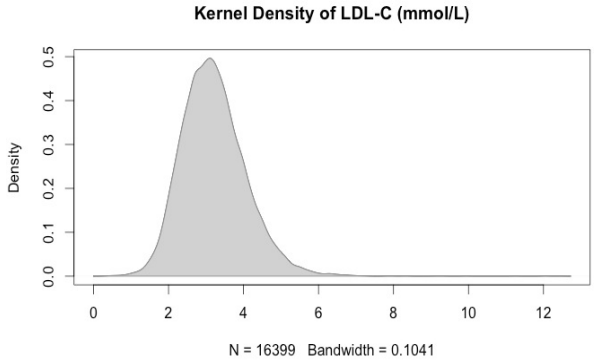
method = "ML",correlation = corCAR1(form = ~ age| subject),data = LDLgrsdata,na.action = na.omit,control=lmeControl(opt="optim"))

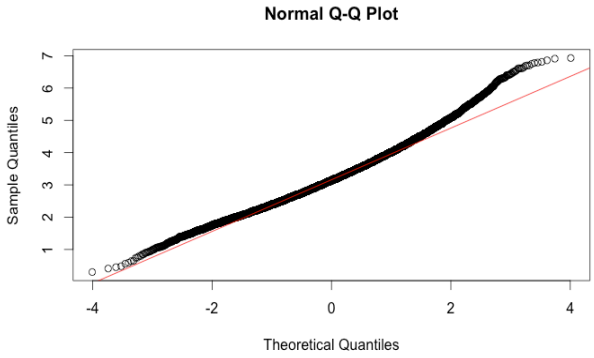
Final model check and validation

Each final IGC model was checked using graphic model validation tools for mixed-models described in Zuur et al [128].

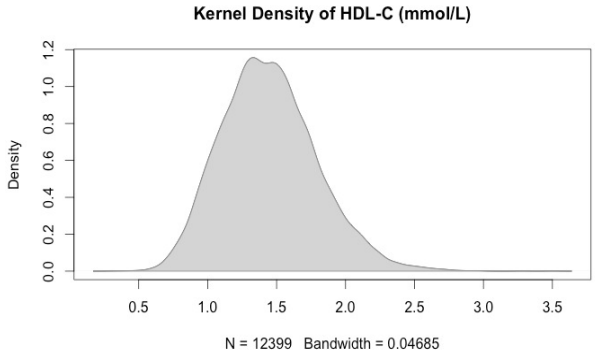
Appendix 3.9. Kernel density plots and quantile-quantile plots of lipoprotein concentrations.

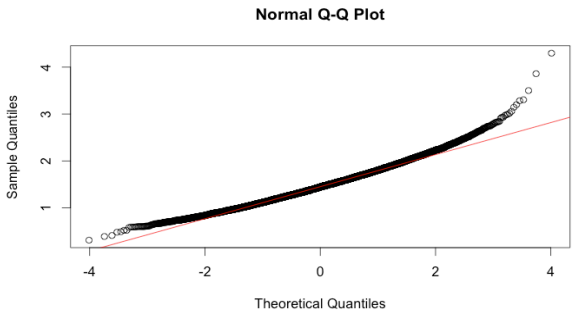
LDL-C levels (mmol/L):





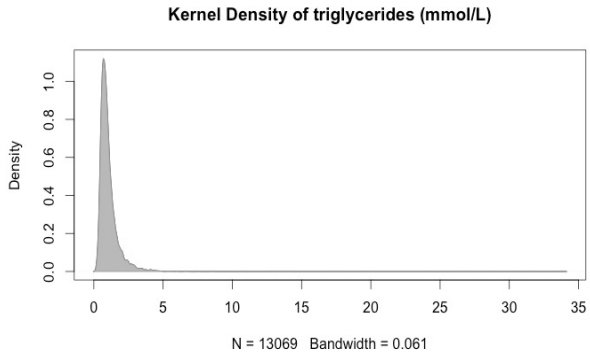
HDL-C levels (mmol/L):

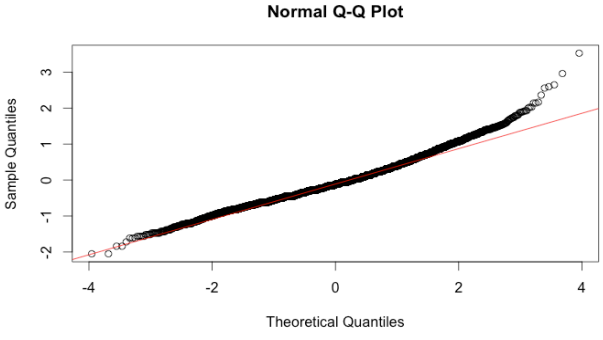




Triglyceride levels (mmol/L):

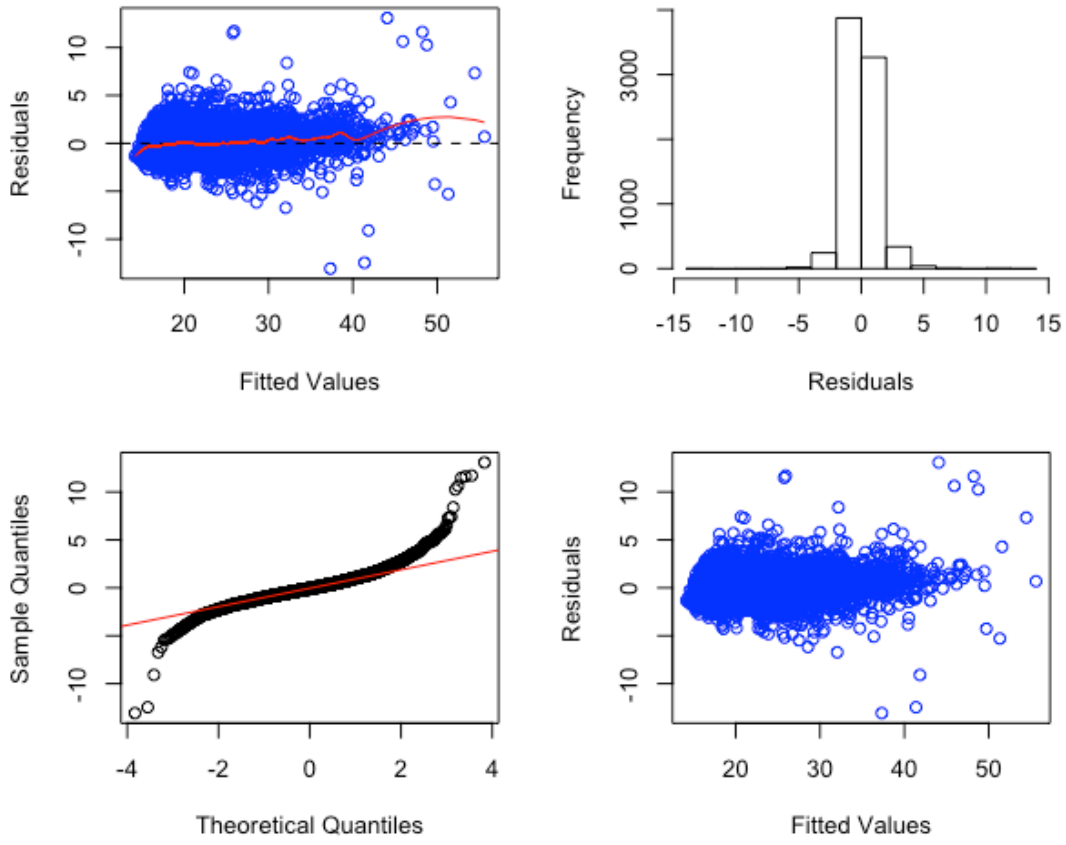
(Before log transformation)





QQ plot of triglycerides distribution after log transformation.

Appendix 3.10. Model Validation plots for the best fitting IGC model. (i) residuals vs. fitted values (homogeneity check), (ii) histograms and QQ-plot of the residuals (normality check), (iii) residuals vs. age (in years) (independence check).



Appendix 4.1. Subset of the YFS cohort used for the BMI trajectory analysis. Reported are the total number (No.) of participants seen at each clinic year, and their ages (Fig 1.), Density plot of the number of BMI measures per YFS participant in the subset of the cohort used for the BMI trajectory analysis (Fig 2.), BMI profiles in 44 randomly selected YFS participants (Fig 3.) and average BMI values in kg/m^2 at each age stratified by T2DM group (pink, no adult T2DM; blue, adult T2DM), with error bars representing the mean BMI ± SD (standard deviation) (Fig 4.).

Fig 1.

Year	**No.**	**YOB[a]**	77	74	71	68	65	62						
1980	2149	**Ages**	3*	6	9	12	15	18						
1983	2149			6	9	12	15	18	21					
1986	1970				9	12	15	18	21	24				
2001	2167						24	27	30	33	36	39		
2007	2058							30	33	36	39	42	45	
2011	1890								34	37	40	43	46	49

[a]YOB (year of birth), corresponds to the 6 birth cohorts represented in the study sample.

*Observations made on 3 years old were excluded from the analyses.

Fig 2.

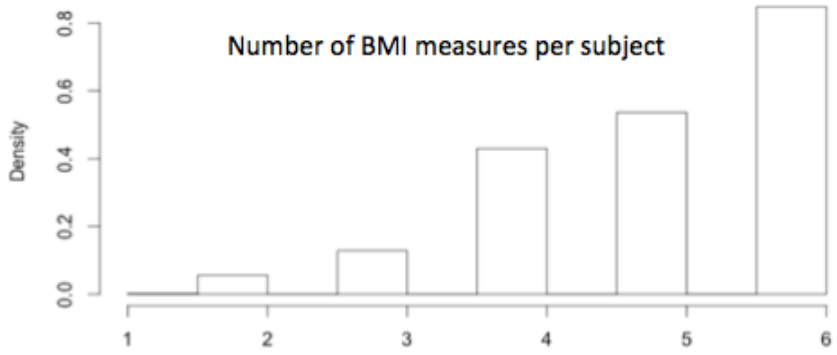
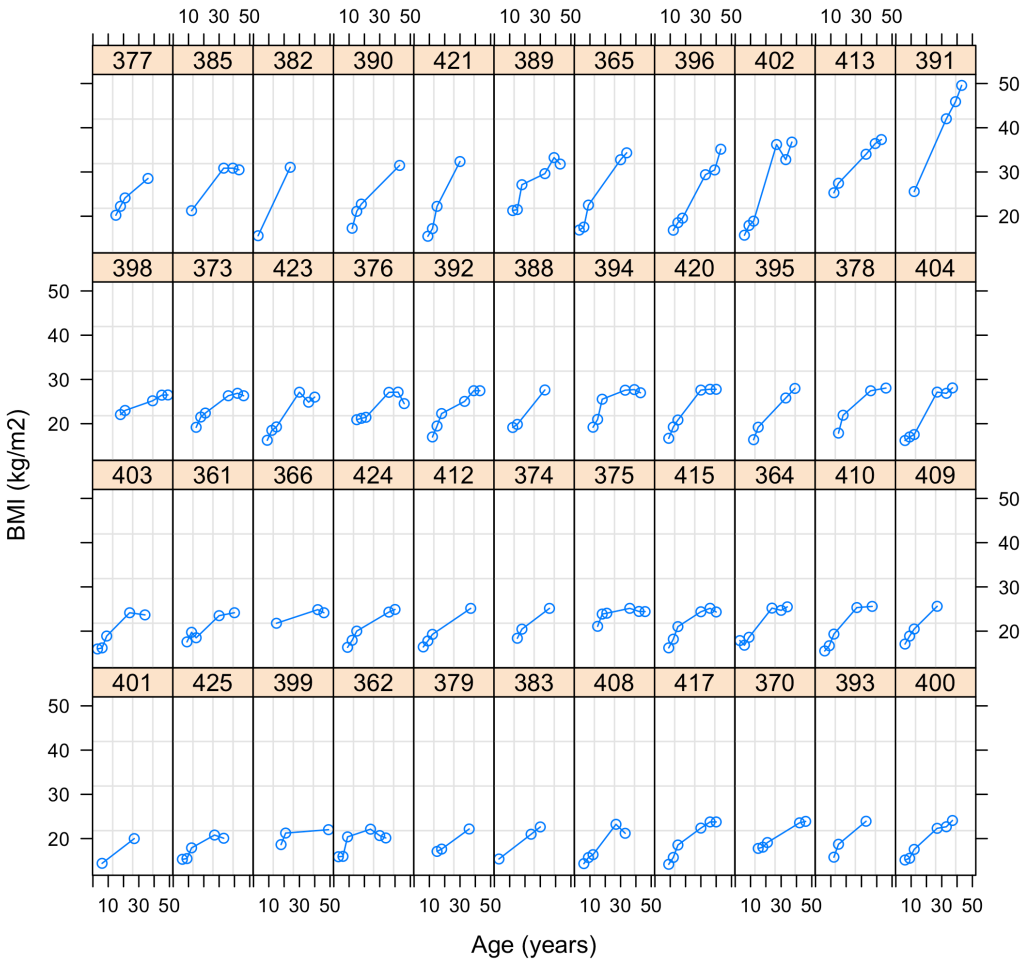
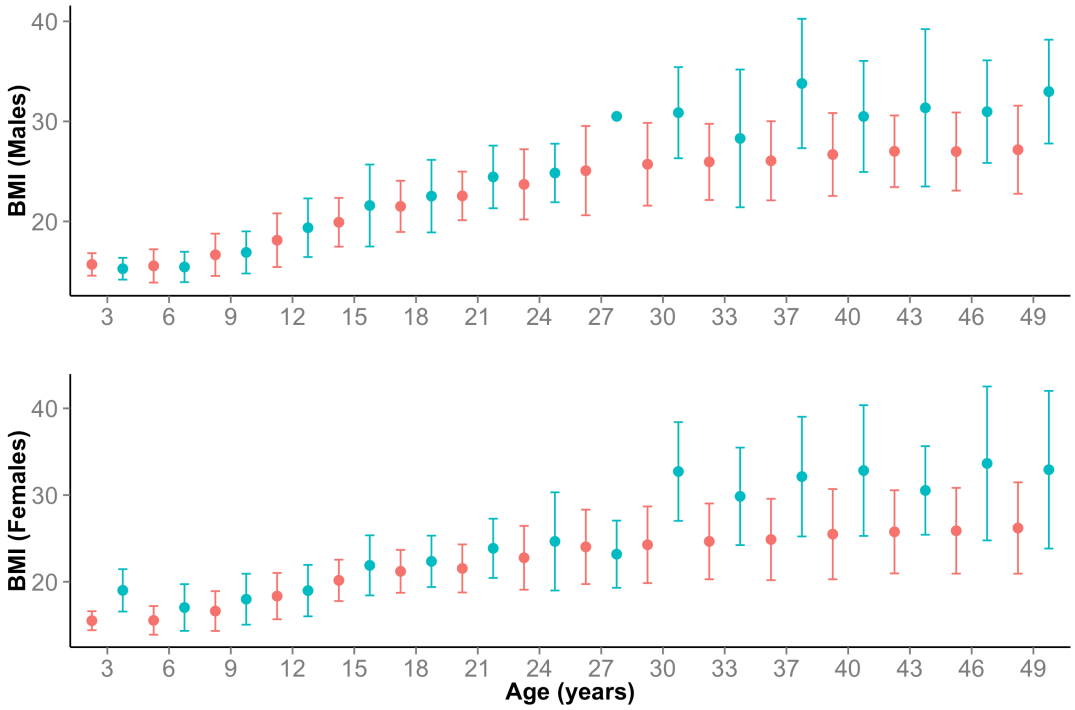


Fig 3.



Values in each panel indicate the ID number of each randomly chosen participant.

Fig 4.


BMI (Males)
40
30
20
3
6
9
12
15
18
21
24
27
30
33
37
40
43
46
49
BMI (Females)
40
30
20
3
6
9
12
15
18
21
24
27
30
33
37
40
43
46
49
Age (years)

Appendix 4.2. Additional information on collection of BMI data, T2DM status and fasting insulin in the YFS subset used in the illustrative analyses in chapter 4.

The YFS is an ongoing population-based prospective cohort that started in Finland in 1980 to investigate cardiovascular risk factors and their determinants from childhood to adulthood, with participants ranging from age 3 to 49 [62]. Between 1980 and 2011, along with other cardiovascular markers of interest, BMI, calculated as BMI = weight (kg) / [height (m)]^2, was collected on a varying number of participants from the original cohort (consisting of 3596 children aged 3 to 18 years in 1980), with up to 6 follow-up measurements per participants: 3 in childhood/young adulthood, and 3 in middle adulthood (Fig 1. and Fig 3. in Appendix 4.1). Fasting Insulin was also measured up to 6 times per participant using methods as previously described [292].

For the calculations of BMI, standing height was measured to the nearest 0.5 cm using a wall-mounted Seca anthropometer, with the participant in bare feet. Weight was measured with participants in light clothes without shoes using bathroom digital Seca weighing scale that recorded to the nearest 0.1 kg.

T2DM status in adulthood was determined at adult follow-ups (2001, 2007, and 2011) as any of: a fasting plasma glucose ≥7 mmol/L (in the absence of type 1 diabetes mellitus), a haemoglobin A1c ≥6.5% (48 mmol/mmol), self-reported in questionnaires of a diagnosis made by a physician, or currently taking an oral glucose-lowering medication. In the analysis, T2DM was used as a dichotomous outcome (i.e. dummy variable coded 0 for participants who remained healthy, and 1 for those with T2DM at their latest adult follow-up, either 2001, 2007 or 2011.

Participants or their parents provided informed consent, and the study was approved by local ethics committees in agreement with the Declaration of Helsinki.

Appendix 4.3. Results of pairwise comparison of Least-square means of BMI in each group (No adult T2DM vs. adult T2DM) at each age

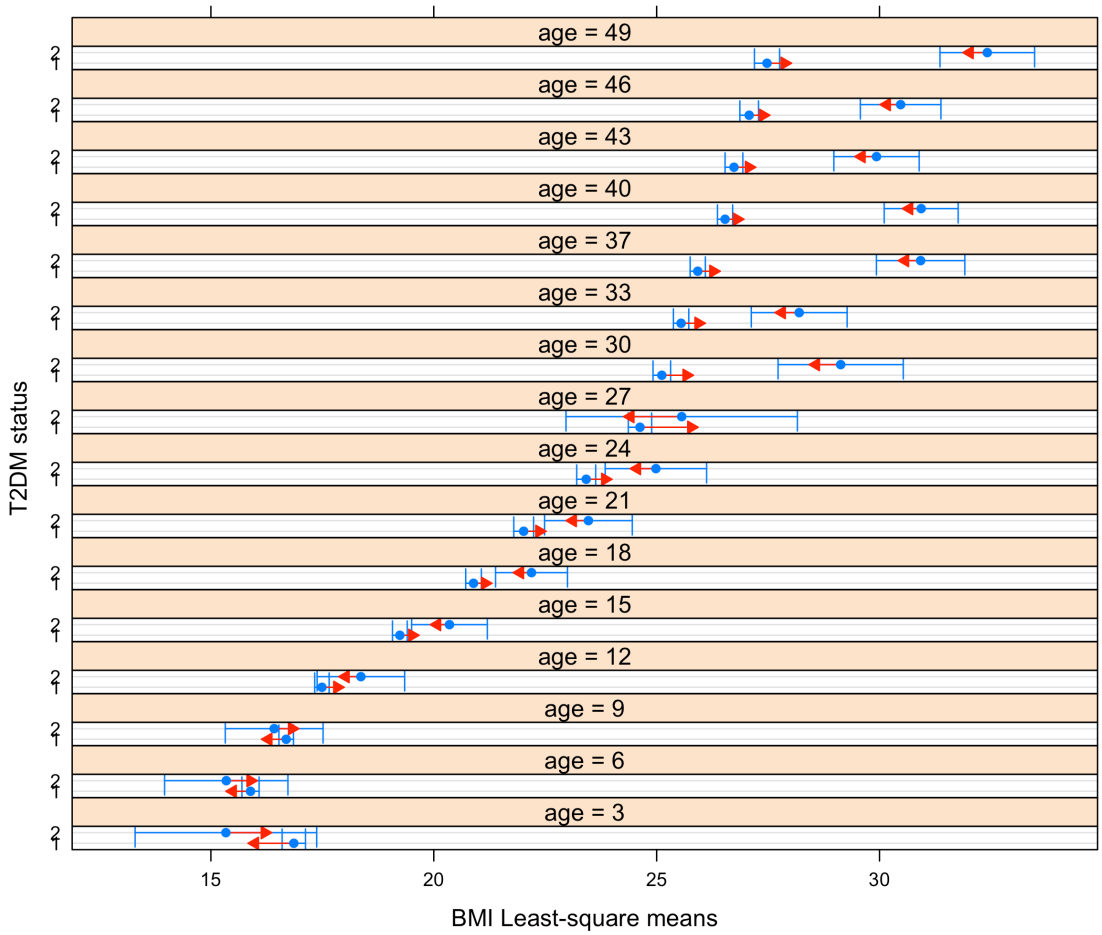
Results of mixed models with age as a categorical predictor and log-insulin as a continuous predictor: LS means contrasts (No adult T2DM vs. adult T2DM) and significance at each age averaged over (**Table 1**), or adjusted for (**Table 2**), the levels of sex, and pairwise comparisons of Least-square means of BMI and 95% CIs at each age in each T2DM status group averaged over-sex (**Fig 1**), and adjusted for status group and sex group combination (M=males, F-females, 1 = No adult T2DM, 2= adult T2DM) (**Fig 2**) and adjusted for *log(insulin)*.

Table 1.Estimated LS means contrasts (in $kg.m^2$) at each age averaged over the levels of sex.

Age (years)	**Estimate*** (kg/m^2)	**SE**	**t-ratio**	**p-value**
3	1.52	1.04	1.455	0.14
6	0.54	0.71	0.76	0.44
9	0.26	0.56	0.47	0.63
12	-0.87	0.50	-1.72	0.0851
15	-1.11	0.44	-2.52	**0.0115**
18	-1.30	0.42	-3.05	**0.002**
21	-1.45	0.51	-2.8	**0.005**
24	-1.56	0.59	-2.64	**0.008**
27	-0.93	1.33	-0.70	0.48
30	-4.01	0.72	-5.54	**<.0001**
33	-2.62	0.55	-4.77	**<.0001**
37	-5.00	0.51	-9.73	**<.0001**
40	-4.39	0.43	-10.17	**<.0001**
43	-3.19	0.49	-6.4	**<.0001**
46	-3.39	0.47	-7.17	**<.0001**
49	-4.94	0.56	-8.81	**<.0001**

* Estimated contrast is obtained from a log(insulin) adjusted model (degrees of freedom is equal to 2339)

Fig 1. Pairwise comparisons of LS means of BMI (in kg.m^2) at each age in each T2DM status group averaged over-sex

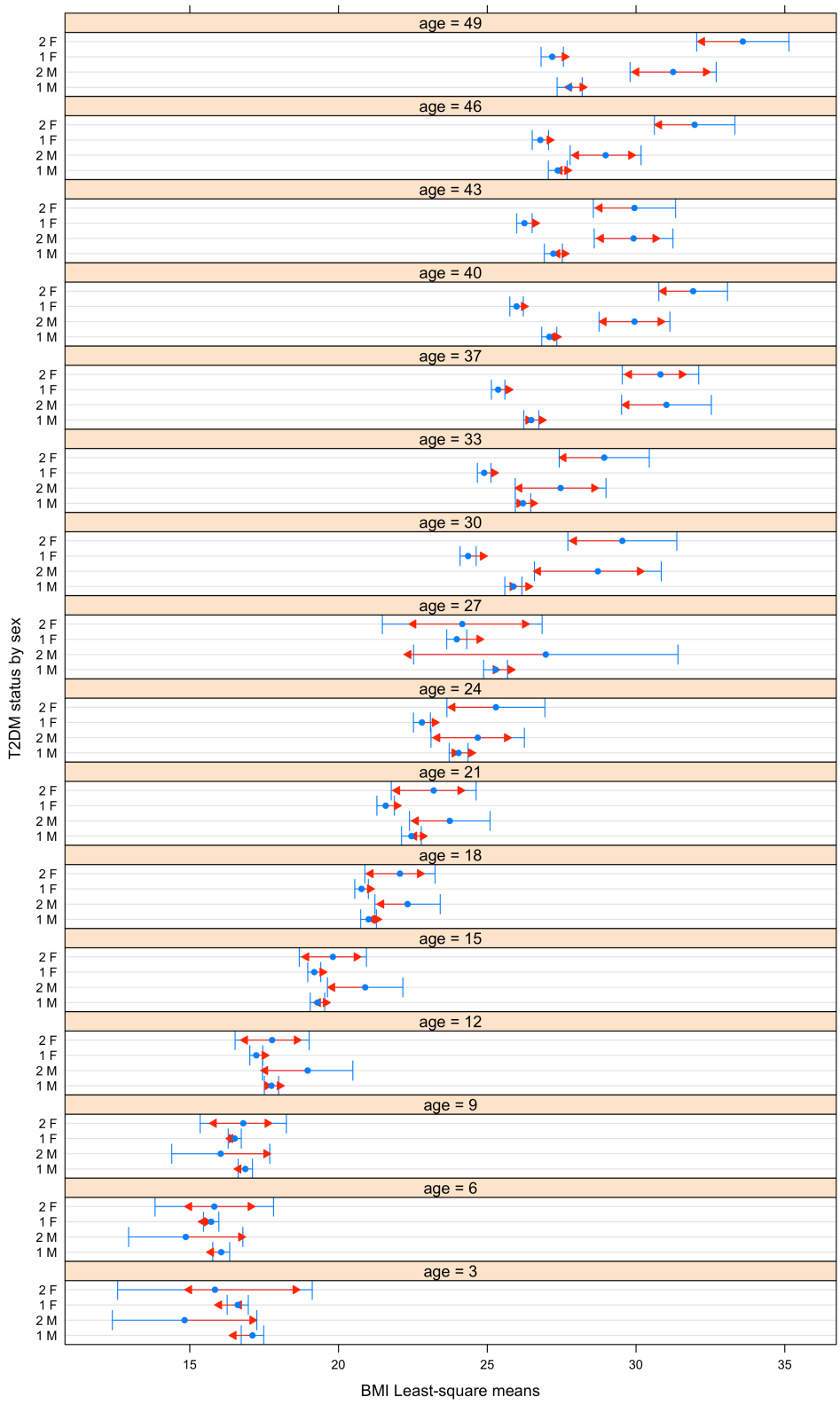


Blue intervals represent the 95% CI for each predicted BMI LS-mean (in kg.m^2). Red comparisons arrows show which LS-means differ significantly from one another based on whether or not they overlap. Comparisons are made using a 0.05 Tukey-adjusted significance level (1: non T2DM group, and 2: T2DM group).

Table 2. Estimated LS means contrasts at each age adjusted for sex.

Age (years)	**Estimate** (kg/m^2)	**SE**	**t-ratio**	**p-value**
Females				
3	0.76	1.67	0.456	0.64
6	-1.01	1.01	-0.10	0.91
9	-0.28	0.74	-0.37	0.70
12	-0.53	0.64	-0.87	0.41
15	-0.6	0.58	-1.07	0.28
18	-1.29	0.61	-2.1	**0.03**
21	-1.61	0.74	-2.2	**0.003**
24	-2.48	0.85	-2.9	**0.003**
27	-0.18	1.37	-0.13	0.89
30	-4.01	0.94	-5.54	**<.0001**
33	-2.62	0.55	-5.1	**<.0001**
37	-5.45	0.78	-8.1	**<.0001**
40	-5.93	0.60	-9.8	**<.0001**
43	-3.69	0.71	-5.15	**<.0001**
46	-5.18	0.4	-7.35	**<.0001**
49	-6.4	0.81	-7.87	**<.0001**
Males				
3	2.28	1.35	1.82	0.06
6	1.19	0.99	1.20	0.22
9	0.82	0.84	0.97	0.33
12	-1.21	0.65	-1.50	0.12
15	-1.60	0.44	-2.43	**0.015**
18	-1.31	0.57	-2.27	**0.03**
21	-1.28	0.71	-1.8	0.06
24	-0.64	0.81	-0.78	0.43
27	-1.68	2.23	-0.74	0.47
30	-2.81	1.09	-2.51	**0.009**
33	-1.26	0.78	-1.6	0.1097
37	-4.50	0.41	-5.83	**<.0001**
40	-2.9	0.68	-4.6	**<.0001**
43	-3.19	0.69	-3.8	**<.0001**
46	-3.39	0.62	-2.5	**0.01**
49	-3.47	0.76	-4.51	**<.0001**

Fig 2. Pairwise comparisons of LS means of BMI (in kg.m^2) at each age in each T2DM status group for each sex.



Blue intervals represent the 95% CI for each predicted BMI LS-mean (in $kg.m^2$). Red comparisons arrows show which LS-means differ significantly from one another based on whether or not they overlap. Comparisons are made using a 0,05 Tukey-adjusted significance level. (1: non T2DM group, 2: T2DM group, F: females, and M: males).

Appendix 4.4. Log-likelihood profiling method and R-code for the choice of priors of the change point mean (μ_{cp}).

When investigating the effect of prior choice (Appendix 2) on the posterior parameters estimates when fitting the unconditional BMI trajectory model, the priors considered for the change point mean in the set 'prior 3', was based on the estimate of the fixed change points for each sex, based on profile-log likelihood in the maximum likelihood. The procedure passes the break point as a model parameter, and minimises the deviance of the fitted model conditional upon the break point using the optimize function in R. This maximizes the profile log likelihood for the break point, and the function interior to the wrapper (lmer() in this case) finds the maximum likelihood estimates conditional upon the change point, so the procedure finds the joint maximum likelihood estimates for all parameters. The following R code was used for each sex to generate the population-average estimates of the knots in the unconditional BMI trajectory model:

```
library(lme4)
# NOTE: In this formulation, intercept is expected BMI value at the
change point itself
#### Estimation of sex-specific average breakpoint based on deviance
minimisation:
#Basis functions
bp = 4
b1 <- function(x, bp) ifelse(x < bp, bp - x, 0)# before slope
b2 <- function(x, bp) ifelse(x < bp, 0, x - bp) # after slope

# Wrapper for Mixed effects model with variable break point
foo <- function(bp)
{
  mod <- lmer(bmi ~ b1(age, bp) + b2(age, bp) + (b1(age, bp) +
b2(age, bp) | id), data = T2DMdataF)
  deviance(mod)
```

```
}
search.range <- c(min(T2DMdataF$age)+0.5,max(T2DMdataF$age)-0.5) #
Vector of Possible knots (=the potential ages at which the
transition in growth rate occurs)

foo.opt <- optimize(foo, interval = search.range)
bp <- foo.opt$minimum # breakpoint that minimises deviance
print(bp)#=16.328 year for females (22 years for males)
```

Appendix 4.5. Prior sensitivity analyses methods and results.

Convergence could not be reached for some parameters in initial analyses of the sex-specific unconditional hierarchical BMI change point models with unrestricted covariance structure between all four random parameters (males and females BMI profiles were analysed separately). As explained in section 3, it was chosen to restrict each model with a correlation between individual childhood slopes (β_1) and adult slopes (β_2), and null correlations between other random effects leading to a block diagonal structure of the variance covariance matrix:

$$\beta_{12} \sim MVN\{\beta_{12}^*, H_{12}\}$$

$$\beta_0 \sim N(\beta_0^*, H_0)$$

$$\Sigma_{12} \sim \text{invWishart}\{\Sigma_{12}^*, 2\}$$

$$\tau_{b0} \sim \text{Gamma}\ (\lambda_{01}, \lambda_{02})$$

$$\tau_i \sim \text{Gamma}\ (\lambda_1, \lambda_2)$$

$$\tau_{CP} \sim \text{Gamma}\ (\lambda_{CP1}, \lambda_{CP2})$$

With:

H_0 the variances for the overall intercept (i.e BMI at 25 years), and H_{12} the covariance matrix for $\beta_{12} = (\beta_{1.}, \beta_2)$. τ_{b0} τ_i τ_{CP} are the precisions (1/variance) for each corresponding parameter (i.e. variances are inverse gamma distributed). Sex specific unconditional models with this block diagonal structure yield lower AIC compared to models with mutually independent random effects, indicating this was an appropriate way to represent the variance structure of the random effects (data not shown here). For the choice of priors for the change point means three potential distributions were considered:

- Normal ($CP_i \sim N(\mu_{CP}^*, H_{CP})$), with μ_{CP}^* and H_{CP} the mean and variance for the change point, respectively. (with μ_{CP}^* =0, $H_{CP} = 1000$).

- Exponential ($CP_i \sim Exp(\lambda)$), where the change point mean is 1/ λ and the change point variance is 1/ λ^2. (we chose $\lambda = 0.05$ ($i.e.\frac{1}{17}$, lowest expected age at transition).
- Uniform ($CP_i \sim uniform(a, b)$), where the mean is b+a/2 and the variance is (b-a)^2/12. (we chose a= -16 (i.e 9-25),b=25 (i.e. 50-25), as ages range between 9 and 50 years and are centered around the grand mean age of 25 years).

The two sex-specific unconditional growth models were fitted with each of these three priors for the change point means (and the priors above for the other model parameters). For both sex the lowest AICs were obtained for $CP_i \sim N(\mu^*_{CP}, H_{CP})$), suggesting that the uniform and exponential distributions are not the best distributions for the change point means (data not shown). As a result, only the prior $CP \sim N(\mu^*_{CP}, H_{CP})$ was considered for the change point means in all subsequent analyses.

Additionally, to investigate the sensitivity of the unconditional trajectory model's results to the choice of the different hyperparameters, three sets of hyperparameters were considered (Table 1):

Table 1. Three different sets of hyperparameters used for the prior sensitivity analysis.

Hyperparameter	Priors 1	Priors 2	Priors 3
β_0^*	0	15	25
β_{12}^*	(0,0)	(0.5,0.2)	(0,0)
μ_{CP}^*	0 for both sexes	10 for both sexes	16 for females 21 for males
H_0	10	100	100
H_{12}	$\begin{pmatrix}10 & 0\\ 0 & 10\end{pmatrix}$	$\begin{pmatrix}10 & 0\\ 0 & 10\end{pmatrix}$	$\begin{pmatrix}100 & 0\\ 0 & 100\end{pmatrix}$
H_{CP}	10	100	100
Σ_{12}^*	$\begin{pmatrix}10 & 0\\ 0 & 10\end{pmatrix}$	$\begin{pmatrix}1 & 0\\ 0 & 1\end{pmatrix}$	$\begin{pmatrix}0.1 & 0\\ 0 & 0.1\end{pmatrix}$
λ_{01}	0.01	0.1	0.001
λ_{02}	0.01	0.1	0.001
λ_{CP1}	0.1	0.01	0.001
λ_{CP2}	0.1	0.01	0.001
λ_1	0.1	0.01	0.001
λ_2	0.1	0.01	0.001
AIC Males	20130	19995	19716
AIC females	28756	27405	26910

The posterior distributions of the model parameters did not have a lot of influence on the marginal posterior distributions of the unconditional model parameters (see Table 2 and Table 3 of estimated parameters for males and females below). The set of priors 'priors 3' was used in all subsequent conditional analyses as they showed the lowest AIC, and also significantly reduced computation time.

Table 2. Estimated parameters for the three considered sets of priors for females.

Parameters Females	**Prior 1**	**Prior 2**	**Prior 3**
β_0	27.25 (0.17)	25.91 (0.47)	26.45 (0.17)
β_1	0.63 (0.01)	0.58 (0.03)	0.61 (0.01)
β_2	-0.49 (0.014)	-0.43 (0.01)	-0.45 (0.014)
CP	16.71 (0.2)	15.95 (0.3)	16.01 (0.2)
$\sigma_{\beta 0}$	2.44 (0.05)	2.68 (0.06)	2.12 (0.05)
$\sigma_{\beta 1}$	0.026 (0.005)	0.014 (0.004)	0.02 (0.003)
$\sigma_{\beta 2}$	0.15 (0.005)	0.16 (0.007)	0.19 (0.005)
σ_{CP}	3.34 (1.14)	4.79 (1.21)	3.12 (1.07)
$\sigma_{\beta 1 \beta 2}$	0.09 (0.24)	0.14 (0.25)	0.10 (0.30)
σ	1.60 (0.011)	1.66 (0.02)	1.42 (0.015)

Table 3. Estimated parameters for the three considered sets of priors for males.

Parameters Males	**Prior 1**	**Prior 2**	**Prior 3**
β_0	27.25 (0.17)	25.91 (0.47)	26.510 (0.17)
β_1	0.63 (0.01)	0.58 (0.03)	0.62 (0.011)
β_2	-0.44 (0.05)	-0.45 (0.04)	-0.48 (0.02)
CP	21.30 (0.62)	22.95 (0.53)	21.83 (0.46)
$\sigma_{\beta 0}$	2.44 (0.05)	2.68 (0.05)	2.38 (0.06)
$\sigma_{\beta 1}$	0.062 (0.005)	0.054 (0.004)	0.07 (0.004)
$\sigma_{\beta 2}$	0.08 (0.007)	0.08 (0.009)	0.05 (0.01)
σ_{CP}	3.34 (1.14)	4.79 (1.21)	5.79 (0.21)
$\sigma_{\beta 1 \beta 2}$	0.017 (0.20)	0.13 (0.22)	0.14 (0.25)
σ	1.50 (0.013)	1.36 (0.02)	1.21 (0.02)

Appendix 4.6. Annotated RJAGS sample code to fit a type 1 trajectory divergence model with a fully unstructured 4 by 4 covariance matrix for the random growth parameters

```
Library(rjags)
Library(mvtnorm)

#######################################
### Type 1 divergence Bayesian model :
#######################################

cat("
    data
{
    # Zero means for random effect on the 4 growth parameters:
    zero.u[1] <- 0 #mean ranef intercept
    zero.u[2] <- 0 #mean ranef slope before CP
    zero.u[3] <- 0 #mean ranef slope AFTER CP
    zero.u[4] <- 0 #mean ranef CP timing
}

    model
{
    # Random intercept,slopes and CP for each subject:
    for( j in 1:M ) #
{
    u[j,1:4] ~ dmnorm(zero.u[1:4],invSigma.u[1:4,1:4])# invSigma.u =
full 4 x 4 precision matrix for random effects

}

    # Define likelihood for each observational unit:
    for( k in 1:N )

{

    # Linear-linear piecewise model (b2 switches off once b3
switches on) :
    mu[k] <- b[1]+u[id[k],1]+(b[2]+u[id[k],2])*Age[k]*(1-
step(Age[k]-(CP+betaGroup*(Grp[k]-1))-u[id[k],4]))
```

```
    +(b[3]+u[id[k],3])*step(Age[k]-CP-betaGroup*(Grp[k]-1)-
u[id[k],4])*(Age[k]-CP-betaGroup*(Grp[k]-1)-u[id[k],4])
    +b[2]*(CP+betaGroup*(Grp[k]-1)+u[id[k],4])*step(Age[k]-CP-
betaGroup*(Grp[k]-1)-u[id[k],4])
    y[k] ~ dnorm(mu[k],tau.e)

    # Other formulatiOn of Random CP (b2 = common slope, b3 =change
to b2 after CP)
    # mu[k] <-
b[1]+u[id[k],1]+(b[2]+u[id[k],2])*Age[k]+(b[3]+u[id[k],3])*step(Age[
k]-CP[Grp[k]]-u[id[k],4])*(Age[k]-CP[Grp[k]]-u[id[k],4])
    #y[k] ~ dnorm(mu[k],tau.e)

}

    # Fixed intercept, slopes and CP (uninformative)
    b[1] ~ dnorm(0.0,1.0E-5)
    b[2] ~ dnorm(0.0,1.0E-5)
    b[3] ~ dnorm(0.0,1.0E-5)
    CP~ dnorm(16.0,1.0E-5)# CP group 1
    betaGroup~ dnorm(0.0,1.0E-5) # difference to CP in group2

    # Residual variance
    tau.e ~dgamma(0.01,0.01)
    sigma.e <-1/1/sqrt( tau.e )

    # Define 4x4 variance-covariance matrix of subject ranefs
    #u[j,1:4] ~ dmnorm(zero.u,invSigma.u)
    invSigma.u[1:4,1:4] ~ dwish(R[1:4,1:4],4) # precision matrix
    Sigma.u[1:4,1:4]<-inverse(invSigma.u[1:4,1:4])# var-cov matrix
    Sigma.u1<-Sigma.u[1,1] # variance intercept
    Sigma.u2<-Sigma.u[2,2] # variance first slope
    Sigma.u3<-Sigma.u[3,3]# variance second slope
    Sigma.u4<-Sigma.u[4,4] ## variance CP
    # correlations
r12<-Sigma.u[1,2]/sqrt(  Sigma.u1*  Sigma.u2)
r13<-Sigma.u[1,3]/sqrt(  Sigma.u1*  Sigma.u3)
r14<-Sigma.u[1,4]/sqrt(  Sigma.u1*  Sigma.u4)
r23<-Sigma.u[2,3]/sqrt(  Sigma.u2*  Sigma.u3)
r24<-Sigma.u[2,4]/sqrt(  Sigma.u2*  Sigma.u4)
```

```
r34<-Sigma.u[3,4]/sqrt(  Sigma.u3*  Sigma.u4)



}",file="otherCPmodel.jag")

############################################################
### fit models
############################################################
track.variables<-c("b","CP"
,"betaGroup","sigma.e","Sigma.u1","Sigma.u2","Sigma.u3","Sigma.u4",
                   "r12","r13","r14","r23","r24","r34")

head(MCdata)

inits=list(b=c(26,0.5,-
0.5),CP=16,betaGroup=0,tau.e=100,invSigma.u=matrix(c(10,0,0,0,0,0.1,
0,0,0,0,0.1,0,0,0,0,10),nrow=4))

R<- matrix(c(0.1,0,0,0,0,0.1,0,0,0,0,0.1,0,0,0,0,0.1),nrow=4)
data=  list("y" = MCdata$mu,
            "Age" = MCdata$age-25,
            "id" =  as.integer(MCdata$id),
            "Grp" = as.integer(MCdata$groupnames),
            "N" = as.integer(nrow(MCdata)),
            "M" = as.integer(length(unique(MCdata$id))),
            "R"=R)

# fit model :
otherCPmodel <- jags.model(
  file = "otherCPmodel.jag",
  data = data,
  inits=inits,
  n.chains = 4,
  n.adapt = 500)

update(otherCPmodel,1000)

sampleotherCPmodel<- coda.samples(otherCPmodel,
                           var = track.variables,
```

```
                              n.iter = 1000,
                              thin = 10 )
summary(sampleotherCPmodel)
```

Appendix 4.7. Function written to retrieve DIC directly from coda.samples() runs in RJAGS

During model estimation, when using the *coda.samples()* function in RJAGS to MCMC sample, it is not usually possible to estimate the DIC from these sample, therefore another run with the *dic.samples()* function is necessary. For complex models that take several hours to run, it can be a severe overhead. The *coda.samples()* source code was thus modified to set up a modified *dic.coda.samples()* function as follows that returns both the DIC and the coda-structure for the MCMC samples.

```
dic.coda.samples <- function (model, variable.names = NULL,
n.iter, thin = 1, ...)
{               load.module('dic') # necessary for pD and
deviance monitor
start <- model$iter() + thin
varnames=c(variable.names, c('deviance', 'pD'))
out <- jags.samples(model, varnames, n.iter, thin,  type =
"trace", ...)               deviance <- out$deviance
 pD <- out$pD
 out$deviance <- NULL
 out$pD <- NULL
 ans <- vector("list", nchain(model))
for (ch in 1: model$nchain()) {
       ans.ch <- vector("list", length(out))
      vnames.ch <- NULL
      for (i in seq(along = out)) {
            varname <- names(out)[[i]]
            d <- dim(out[[i]])
            if (length(d) < 3) {
                  stop("Invalid dimensions for sampled
            output")                                    }
            vardim <- d[1:(length(d) - 2)]
             nvar <- prod(vardim)
             niter <- d[length(d) - 1]
             nchain <- d[length(d)]
            values <- as.vector(out[[i]])
```

```
            var.i <- matrix(NA, nrow = niter, ncol = nvar)
            for (j in 1:nvar) {
                var.i[, j] <- values[j + (0:(niter - 1)) *
          nvar +
          (ch - 1) * niter * nvar]
          }
     vnames.ch <- c(vnames.ch, rjags:::coda.names(varname,
vardim))
     ans.ch[[i]] <-var.i
     }
ans.ch <- do.call("cbind", ans.ch)
colnames(ans.ch) <- vnames.ch
ans[[ch]] <- mcmc(ans.ch, start = start, thin = thin)
}
dic <- list(deviance = mean(as.vector(deviance)), penalty =
mean(as.vector(pD)), type = 'pD')
class(dic) <- "dic"
return(list(samples=mcmc.list(ans), dic=dic))
}
```

Appendix 4.8. Model Validation plots for the best fitting model for females (model E). Similar plots were obtained for males but are not displayed here due to space limitations. Fig (A) Traceplots displaying iterations vs. sampled values for all variables of interest in the 4 chains, use to assess the mixing of the chains, Fig (B) density plots showing the posterior probability density function of all estimated parameters.

Fig (A)

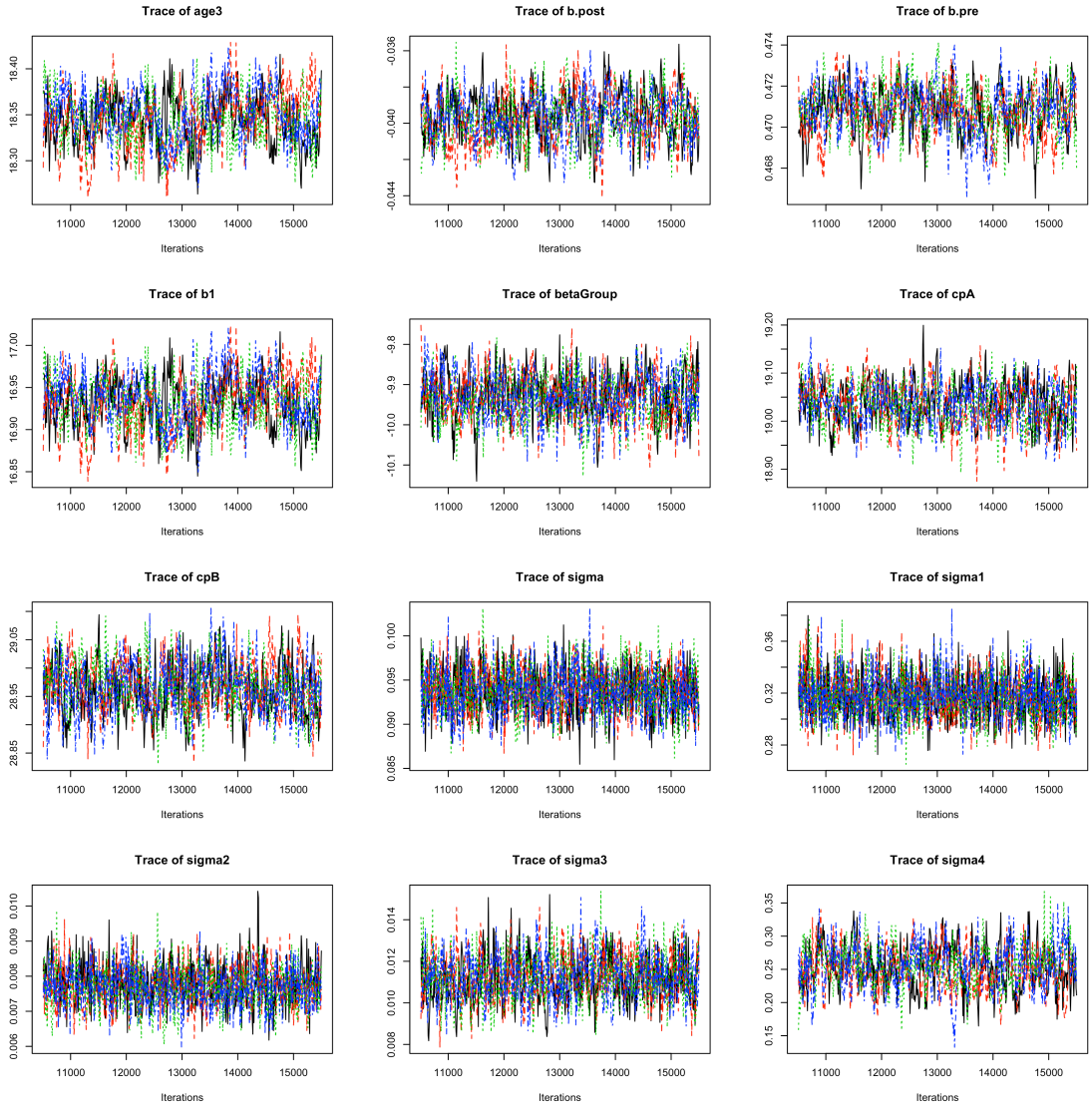
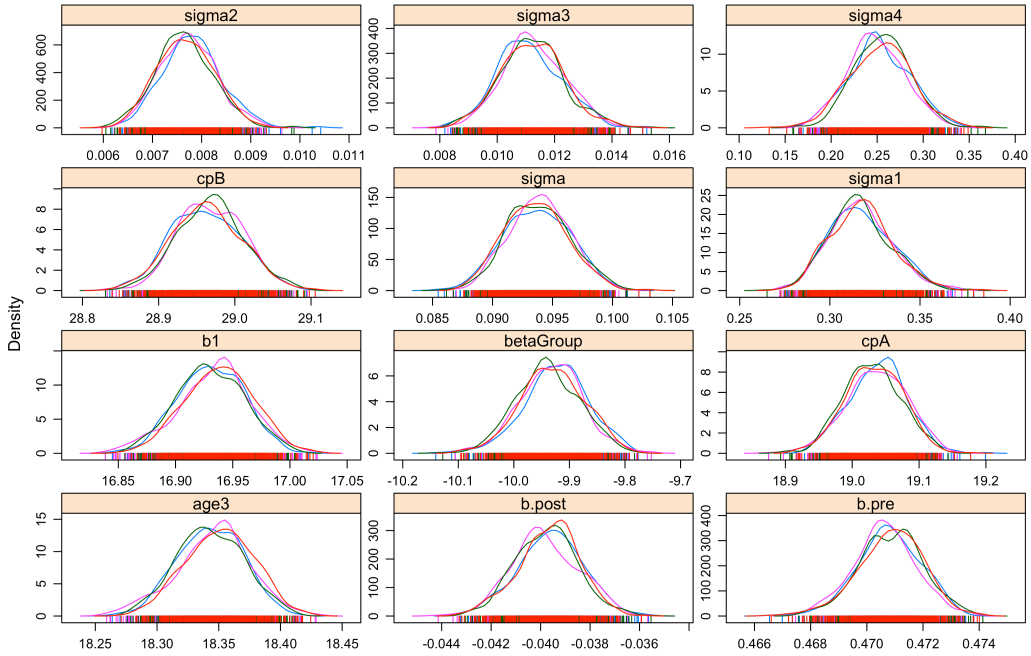


Fig (B)


sigma2
sigma3
sigma4
cpB
sigma
sigma1
b1
betaGroup
cpA
age3
b.post
b.pre
Density

Appendix 4.9. Additional model validation diagnostic statistics obtained for the best fitting model for females (model E): the Geweke, Gelman-Rubin, Heidelberger-Welch, and the Raftery-Lewis diagnostics, respectively.

Number of chains = 4
Number of iterations = 500 per chain before discarding the burn-in period
The burn-in period = 100 per chain
Sample size in total = 1600

********** The Geweke diagnostic: **********
Z-scores:

	chain1	chain 2	chain 3	chain 4
age3	1.87790409	-0.9116443	1.9562705	0.08257904
b.post	0.37392027	1.8108930	0.3126379	0.94049454
b.pre	0.68937062	0.4609849	-1.6319973	0.12470341
betaGroup	0.51433394	-0.5308812	1.6467914	0.15191896
cpA	-0.90531483	-1.0411778	1.4292255	0.86148340
cpB	-1.35870994	-0.4896347	-1.4099419	0.60453047
sigma	0.01517389	0.7292286	1.0197183	-1.17322858
sigma1	-1.71732317	-0.4743896	-0.8881653	0.53244460
sigma2	1.29401726	0.2975920	1.2663673	0.06317473
sigma3	0.08981785	-1.4389448	1.2047930	0.14767469
sigma4	-0.05636982	-1.9683511	0.2852076	1.17909705
Window From Start	0.10000000	0.4683000	0.7741100	0.49450000
Window From Stop	0.50000000	0.3296200	0.0123300	0.23903000

********** The Gelman-Rubin diagnostic: **********
Potential scale reduction factors:

	Point est.	Upper C.I.
age3	1.05	1.14
b.post	1.00	1.01
b.pre	1.02	1.06
betaGroup	1.01	1.03
cpA	1.00	1.00
cpB	1.02	1.05
sigma	1.00	1.00
sigma1	1.00	1.01
sigma2	1.01	1.04
sigma3	1.01	1.02
sigma4	1.05	1.15

Multivariate psrf

1.09

********** The Heidelberger-Welch diagnostic: **********

Chain 1, epsilon=0.1, alpha=0.05

	Stationarity test	start iteration	p-value
age3	passed	1	0.404
b.post	passed	1	0.852
b.pre	passed	1	0.824
betaGroup	passed	1	0.802
cpA	passed	1	0.902
cpB	passed	1	0.585
sigma	passed	1	0.518
sigma1	passed	1	0.846
sigma2	passed	1	0.093
sigma3	passed	1	0.939
sigma4	passed	1	0.987

	Halfwidth test	Mean	Halfwidth
age3	passed	18.34540	7.64e-03
b.post	passed	-0.03953	2.32e-04
b.pre	passed	0.47091	3.83e-04
betaGroup	passed	-9.91759	1.52e-02
cpA	passed	19.03645	1.18e-02
cpB	passed	28.95404	1.04e-02
sigma	passed	0.09374	3.02e-04
sigma1	passed	0.31858	1.93e-03
sigma2	passed	0.00772	6.82e-05
sigma3	passed	0.01113	1.31e-04
sigma4	passed	0.24822	7.98e-03

Chain 2, epsilon=0.058, alpha=0.1

	Stationarity test	start iteration	p-value
age3	passed	1	0.603
b.post	passed	161	0.380
b.pre	passed	1	0.825
betaGroup	passed	1	0.593
cpA	passed	1	0.179
cpB	passed	1	0.426
sigma	passed	1	0.409
sigma1	passed	1	0.136
sigma2	passed	1	0.866
sigma3	passed	1	0.248
sigma4	passed	1	0.280

	Halfwidth test	Mean	Halfwidth

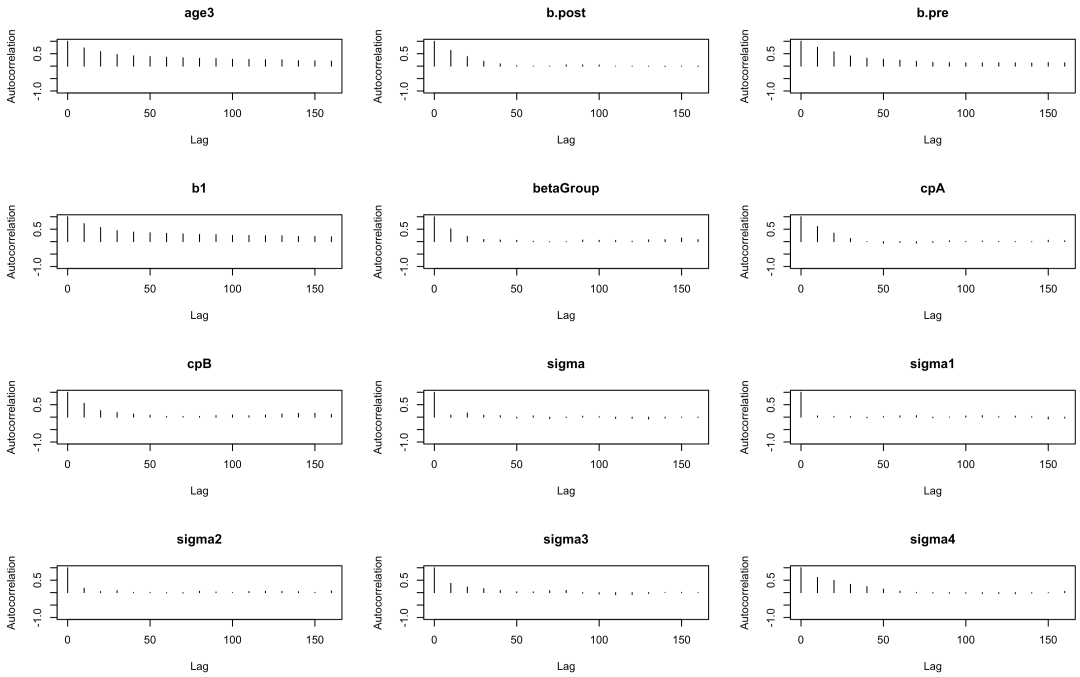
```
        test
age3      passed    18.34950 6.14e-03
b.post    passed    -0.03969 3.83e-04
b.pre     passed     0.47048 2.81e-04
betaGroup passed    -9.93687 8.98e-03
cpA       passed    19.03428 9.92e-03
cpB       passed    28.97114 9.06e-03
sigma     passed     0.09377 3.07e-04
sigma1    passed     0.31778 1.72e-03
sigma2    passed     0.00775 7.33e-05
sigma3    passed     0.01121 1.40e-04
sigma4    passed     0.25132 6.44e-03

Chain 3, epsilon=0.036, alpha=0.025
        Stationarity start     p-value
        test         iteration
age3      passed       1         0.464
b.post    passed       1         0.480
b.pre     passed       1         0.101
betaGroup passed       1         0.104
cpA       passed       1         0.235
cpB       passed       1         0.378
sigma     passed       1         0.385
sigma1    passed       1         0.576
sigma2    passed       1         0.129
sigma3    passed       1         0.474
sigma4    passed       1         0.241

        Halfwidth Mean     Halfwidth
        test
age3      passed    18.34128 8.06e-03
b.post    passed    -0.03959 3.32e-04
b.pre     passed     0.47071 3.82e-04
betaGroup passed    -9.93246 8.71e-03
cpA       passed    19.03534 9.14e-03
cpB       passed    28.96780 9.84e-03
sigma     passed     0.09363 2.54e-04
sigma1    passed     0.31592 1.89e-03
sigma2    passed     0.00764 8.11e-05
sigma3    passed     0.01118 1.73e-04
sigma4    passed     0.26110 6.85e-03

Chain 4, epsilon=0.116, alpha=0.05
        Stationarity start     p-value
        test         iteration
age3      passed       1         0.255
b.post    passed       1         0.374
b.pre     passed       1         0.709
```

```
betaGroup passed       1         0.946
cpA       passed       1         0.310
cpB       passed       1         0.445
sigma     passed       1         0.319
sigma1    passed       1         0.377
sigma2    passed       1         0.833
sigma3    passed       1         0.949
sigma4    passed       1         0.374

          Halfwidth Mean     Halfwidth
          test
age3      passed    18.34039 9.24e-03
b.post    passed    -0.03955 2.06e-04
b.pre     passed     0.47062 2.84e-04
betaGroup passed    -9.92315 1.01e-02
cpA       passed    19.04328 9.31e-03
cpB       passed    28.96643 8.27e-03
sigma     passed     0.09348 2.54e-04
sigma1    passed     0.31756 1.61e-03
sigma2    passed     0.00779 7.21e-05
sigma3    passed     0.01110 1.31e-04
sigma4    passed     0.25037 5.59e-03

********** The Raftery-Lewis diagnostic: **********

Chain 1, converge.eps = 0.001
Quantile (q) = 0.025
Accuracy (r) = +/- 0.005
Probability (s) = 0.95

You need a sample size of at least 3746 with these values of q, r and s

Chain 2, converge.eps = 0.005
Quantile (q) = 0.1
Accuracy (r) = +/- 0.005
Probability (s) = 0.975

You need a sample size of at least 18086 with these values of q, r and s

Chain 3, converge.eps = 5e-04
Quantile (q) = 0.05
Accuracy (r) = +/- 0.001
Probability (s) = 0.95

You need a sample size of at least 182470 with these values of q, r and s

Chain 4, converge.eps = 5e-04
Quantile (q) = 0.25
```

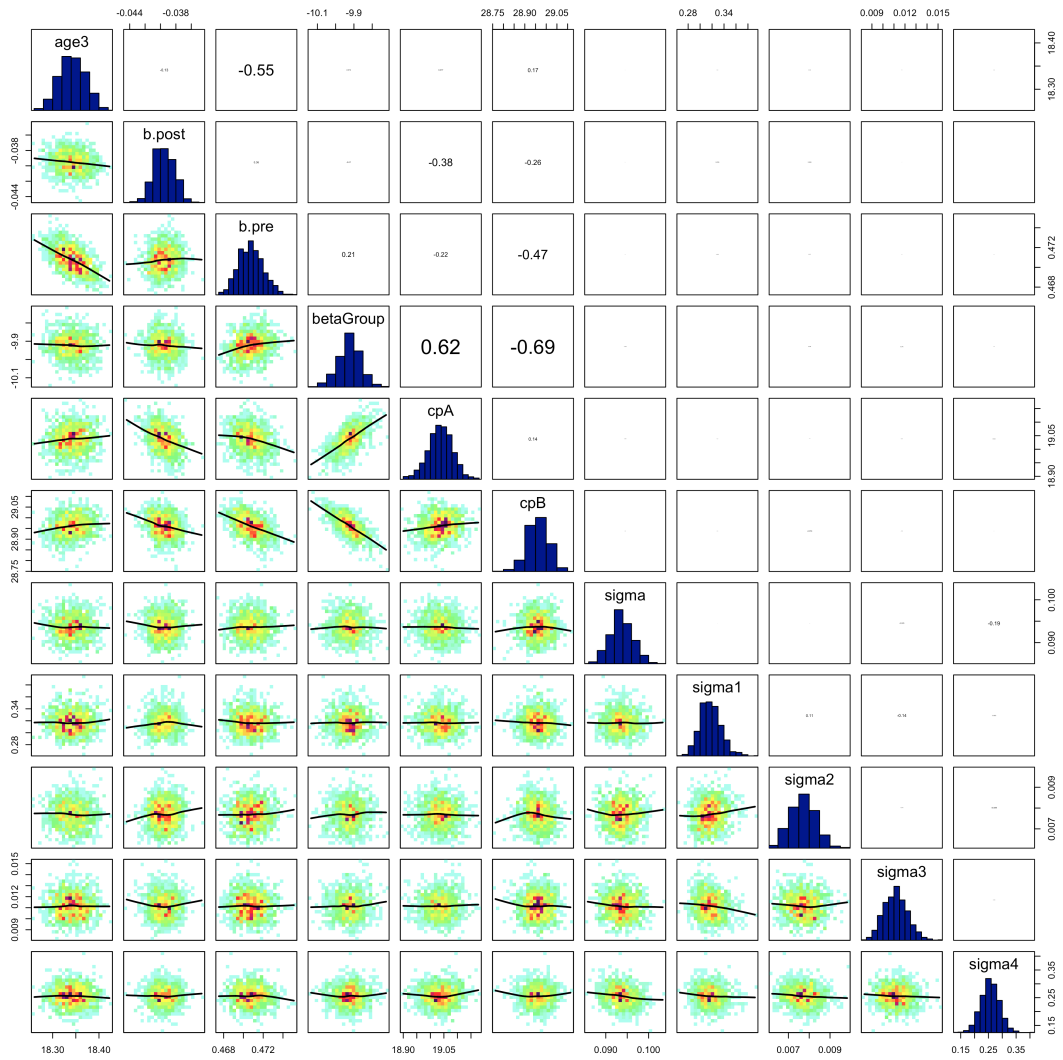
Accuracy (r) = +/- 0.001
Probability (s) = 0.975

You need a sample size of at least 941979 with these values of q, r and s.

Appendix 4.10. Autocorrelation plots for best fitting Model (Model E)



Appendix 4.11 Pairwise correlation plots for parameters estimated in the best fitting Model (Model E) for females: Marginal densities (diagonal), pairwise densities (lower panels) and correlation coefficient (upper panels) for the fit.



Appendix 5.1. Subset of the Cardiovascular Risk in Young Finns study cohort used for the BMI trajectory analysis in Chapter 5 (Table 1), and Number of repeated BMI measures per person for included participants (N_{total} =2717 participants) (Table 2).

Table 1. Subset of the Cardiovascular Risk in Young Finns study cohort used for the BMI trajectory analysis in Chapter 5

Year	No.	Ages														
1980	2717	3*	6	9	12	15	18									
1983	2279		6	9	12	15	18	21								
1986	2036			9	12	15	18	21	24							
1989	317				12	15	18	21	24	27						
1992	504					15	18	21	24	27	30					
2001	2236								24	27	30	33	36	39		
2007	2134										30	33	36	39	42	45
2011	2015											34	37	40	43	46

Reported are; numbers (No.) of participants with non-missing BMI data at each follow-up who also had non-missing childhood adiposity status at baseline (in 1980) and non-missing obesity status at least once in adulthood (either in 2001, 2007, or 2011); and age at each measurement. (*: Observations made at 3 years old (from individuals born in 1977) were excluded from the analyses). The birth year of the six cohorts were 1977, 1974, 1971, 1968, 1965, and 1962

.

Table 2. Number of repeated BMI measures per person for included participants (N_{total} =2717 participants).

	No. BMI measures						
	2	**3**	**4**	**5**	**6**	**7**	**8**
Number of participants	86	185	548	609	907	224	224
Proportion (%) of participants	3.2	6.8	20.2	22.4	33.4	8.3	5.8

Appendix 5.2. Number of participants (%) and mean age (SD) in each adiposity status group stratified by sex.

	Group I (Reference)	**Group II** (Resolving high adiposity)	**Group III** (Persisting high adiposity)	**Group IV** (Incident obese)	**Total**
Number of participants (%)					
Males	961 (76.7%)	35 (2.8%)	73 (5.8%)	183 (14.7%)	1252
Females	1124 (76.7%)	58 (3.9%)	62 (4.2%)	221 (15.1%)	1465
Total	2085 (76.7%)	93 (3.4%)	135 (4.9%)	404 (14.9%)	2717
Mean (SD) age in years					
Males	23.7 (13.1)	24.5 (12.9)	26.0 (13.1)	24.9 (12.9)	24.9 (12.9)
Females	24.3 (13.1)	21.5 (12.5)	25.1 (12.8)	24.8 (13.1)	24.3 (13.0)
Total	24.0 (13.1)	22.6 (12.8)	25.6 (12.9)	24.9 (13.0)	24.6 (13.1)

Appendix 5.3. Marginal posterior means, standard deviation (sd) and density distribution (95% credible interval (CI: (2.5^{th}, 97.5^{th}) percentile) of the average BMI trajectory parameters for each sex (M, males and F, females) and obesity-group (I, II, III and IV) between age 6 and 49 years (i.e. intercept (in kg m^2), childhood slope (S_1, in kg m^2/year), difference between childhood and adult slope (S_2, in kg m^2/year) and change point (CP, in years)).

All parameters estimates in the table are significantly different from zero (none of the reported 95% CI includes zero).

			Males			Females	
Parameter	**Obesity**	**Group**	**Mean (sd)**	**95% CI**	**Group**	**Mean (sd)**	**95% CI**
Intercept*	Reference	I M	23.97 (0.09)	23.73 – 24.14	I F	24.21 (0.11)	(24.00, 24.51)
	Resolving	II M	28.56 (0.6)	27.95 – 30.03	II F	28.02 (0.75)	(27.34, 29.87)
	Persistent	III M	30.5 (1.12)	25.51 – 33.47	III F	31.4 (1.2)	(26.42, 34.80)
	Incident	IV M	28.65 (0.24)	28.14 – 29.07	IV F	27.62 (0.33)	(27.03, 29.12)
S_1	Reference	I M	0.46 (0.008)	0.45 – 0.48	I F	0.48 (0.008)	(0.47, 0.50)
	Resolving	II M	0.48 (0.04)	0.42 – 0.53	II F	0.46 (0.04)	(0.40, 0.50)
	Persistent	III M	0.55 (0.06)	0.43 – 0.60	III F	0.56 (0.06)	(0.45, 0.60)
	Incident	IV M	0.63 (0.01)	0.61 – 0.65	IV F	0.62 (0.01)	(0.60, 0.63)
CP	Reference	I M	24.1 (0.41)	22.9 – 26.0	I F	17.2 (0.34)	(16.1, 18.3)
	Resolving	II M	21.4 (1.74)	19.2 – 24.5	II F	16.1 (1.4)	(14.6, 19.1)
	Persistent	III M	24.7 (3.36)	18.5 – 27.8	III F	27.1 (2.58)	(22.7, 30.2)
	Incident	IV M	30.1 (0.8)	28.6 – 32.3	IV F	29.7 (0.41)	(28.2, 31.2)
S_2**	Reference	I M	-0.40 (0.007)	-0.39 – -0.40	I F	-0.39 (0.009)	(-0.38, -0.39)
	Resolving	II M	-0.44 (0.05)	-0.43 – -0.46	II F	-0.390 (0.03)	(-0.37, -0.41)
	Persistent	III M	-0.33 (0.01)	-0.31 – -0.34	III F	-0.255 (0.01)	(-0.25, -0.27)
	Incident	IV M	-0.55 (0.007)	-0.55 – -0.56	IV F	-0.47 (0.009)	(-0.46, -0.48)

*Reported intercepts correspond to the expected BMI levels (in kg/m^2) at 25 years ("age" variable centered around 25 years in the model) provided they are in the first phase of growth. The intercepts control for the initial BMI level at age 6 years and the predicted BMI levels at 6 years of age for each group (as seen in Fig 5.1. and 5.2) can be retrieved via the youth slopes as follows: $BMI_{age6} = Intercept + S1 * (25 - 6)$.

**S_2 is the difference (i.e. deviation) between adult slopes and childhood slopes (S_1). Adult slopes can thus be calculated as S_1+S_2.

Appendix 5.4. Marginal posterior means, standard deviations (sd), density distribution and significance (*) of the differences in BMI trajectory parameters between sex and adiposity groups.

Parameter	Group differences[d]	Posterior mean (sd)	95% CI	(*)
Intercept [a]	I M-I F	-0.24 (0.16)	(-0.6, 0.07)	
	IV M –IV F	0.44 (0.41)	(-0.3, 1.2)	
	I M- IV M	-0.83 (0.25)	(-0.59, -1.07)	*
	I F- IV F	-0.84 (0.36)	(-0.48, -1.18)	*
	II M-II F	0.5 (0.31)	(-0.09,1.08)	
	III M –III F	-0.9 (0.76)	(-2.30, 0.51)	
	II M- III M	-2.01 (0.63)	(-3.24, -0.77)	*
	II F- III F	-3.4 (0.78)	(-4.5, -1.79)	*
S_1 [b]	I M-I F	-0.02 (0.01)	(-0.039, -4.10^{-4})	*
	IV M –IV F	-0.015 (0.02)	(-0.05, 0.02)	
	I M- IV M	-0.17 (0.03)	(-0.22, -0.11)	*
	I F- IV F	-0.135 (0.04)	(-0.20, -0.06)	*
	II M-II F	0.023 (0.06)	(-0.09, 0.14)	
	III M –III F	-0.01 (0.04)	(-0.08, 0.07)	
	II M- III M	-0.06 (0.03)	(-0.12, -0.01)	*
	II F- III F	-0.09 (0.03)	(-0.15, -0.03)	*
S_2 [b]	I M-I F	-0.007 (0.01)	(-0.02, 0.01)	
	IV M –IV F	-0.008 (0.02)	(-0.04,0.03)	
	I M- IV M	0.08 (0.04)	(-0.001,0.12)	
	I F- IV F	0.15 (0.05)	(0.21,0.04)	*
	II M-II F	-0.055 (0.06)	(-0.16, 0.06)	
	III M –III F	-0.082 (0.03)	(-0.14, -0.02)	*
	II M- III M	-0.11 (0.04)	(-0.18, -0.03)	*
	II F- III F	-0.14 (0.03)	(-0.19, -0.08)	*
CP [c]	I M-I F	6.31 (0.54)	(5.25, 7.36)	*
	IV M -IV F	0.2 (0.08)	(-0.01, 0.33)	
	I M-IV M	-5.89 (1.03)	(-7.9, -3.7)	*
	I F- IV F	-12.06 (2.1)	(-15.1, -6.5)	*
	II M-II F	5.32 (1.26)	(2.81, 7.91)	*
	III M-II F	-2.1 (1.8)	(-5.21, 1.32)	
	II M- III M	-3.6 (0.72)	(-5.23, -2.48)	*
	II F- III F	-2.1 (0.7)	(-15.32, -5.76)	*

Shown in the table are: (1) differences in BMI trajectory parameters (i.e. intercept at age 6, childhood slope (S_1), adult slope (S_2) and change point (CP)) between sexes (Male (M) vs. Females (F)*); and, (2) the differences in BMI trajectory parameters between persistent-non obese and incident obese participants (Group I vs. Group IV*), and between obesity resolving and persistent obese participants (Group II vs. Group III*), for males (M) and females (F) respectively. To help readability, the table cells concerned with the sex differences in parameters for each specific obesity groups are shaded in grey, while the cells concerned with the differences between obesity groups for a given sex are in white.

[a] :Between-group differences in intercept are reported in $Kg.m^2$. For ease of interpretation, intercepts presented in this table are the expected BMI level at 6 years.

[b] : Between-group differences in slopes (S_1 and S_2) are reported in Kgm^2/year.

[c] : Between-group differences in change point timing (CP) are reported in years.

[d] : The differences in parameters are reported as $\beta_{Males} - \beta_{Females}$ and as $\beta_{Reference} - \beta_{Incident}$, or $\beta_{Resolving} - \beta_{Persistent}$, where β represent the four trajectory parameters (Intercepts, slopes S_1 and S2, and change points CP), respectively.

Appendix 5.5. Empirical posterior mean, Bayesian standard deviation (SD) and posterior density quantiles (95% credible interval (CI): 2.5th, 97.5th percentile) for cohort effects on each of the four growth parameters (estimated from the sex and adiposity status adjusted Bayesian hierarchical piecewise regression model).

Parameter *	Mean	SD	95% CI
Int_{yob}	0.0046	0.013	-0.02, 0.003
$S1_{yob}$	-4.12.10^{-4}	0.013	-0.001, 0.002
$S2_{yob}$	0.0004	0.0008	-0.002, 0.001
CP_{yob}	0.038	0.046	-0.05, 0.12

*Each β_{yob} parameter, where β = intercept, S_1, S_2, or CP, can be interpreted as the expected linear change in the corresponding growth parameter for a 1 year increase in calendar year of birth.

Appendix 5.6. Posterior mean parameter estimates, standard deviation (SD) and posterior distribution interval (95% credible interval (CI): 2.5th , 97.5th percentile) for the variance parameters (random effects and error term) of the sex-and adiposity specific Bayesian hierarchical piecewise regression model.

Parameter*	Posterior mean (SD)	95% CI[a]
$\sigma_{intercept}$	1.36 (0.011)	1.34, 1.38
σ_{S1}	1.51 (0.03)	1.45, 1.57
σ_{S2}	0.03 (0.003)	0.024, 0.038
σ_{CP}	0.07 (0.005)	0.065, 0.086
σ	5.04 (0.19)	4.65, 5.38

*σ coefficients are standard deviations of the corresponding growth parameters (intercept, S_1, S_2, and CP) or the residual (level-1) error.

[a] All estimated parameter estimated are significant.

Appendix 5.7. Age- and sex-specific and average prevalence of childhood overweight, obesity and overweight or obesity at baseline in 1980 in the YFS subset used for BMI trajectory analyses (2717 participants).

Baseline Age (years)	No. Participants*	No. Overweight*	No. Obese*	% Overweight or obese*
3	415 (201 / 214)	14 (6 / 8)	1 (0/1)	3.0 (2.9 /4.2)
6	446 (194 / 252)	35(11 / 24)	13 (6/7)	10.3 (8.6 / 12.3)
9	477 (227 / 250)	47 (21 / 26)	6 (3/3)	11.1 (10.5 / 11.6)
12	482 (213 / 269)	39 (18 / 21)	6(3/3)	8.9 (9.8 / 8.9)
15	468 (221 / 247)	25 (13 /12)	3 (3 /0)	5.9 (7.2 / 4.8)
18	429 (196 / 233)	35 (21 / 14)	4 (3 / 1)	8.1 (12.7 / 6.4)
N_{total}	2717 (1252 / 1465)	179 (96/83)	34 (18/16)	8.4 (9.1/ 6.7)

*data are total (males /females)).

Appendix 5.8. Supplementary Methods – Additional information on Bayesian Hierarchical Piecewise Growth model formulation and R code used to fit the models used in chapter 5

Model formulation

Chaper 5 uses the Bayesian hierarchical piecewise regression approach formulated in chapter 4

Consistent with well-established between-sexes differences in the development of BMI between childhood and adulthood in normal weight individuals [308, 309], exploratory analyses in this study suggested sex differences in BMI patterns over age in the YFS sample when participants were stratified according to their obesity resolution status. As in chapter 4, the analyses in chapter 5 did not consider BMI measures from 3 years old in the trajectory analyses, because the low number of follow-up clinics in young children in the YFS prevented to model the BMI turning points of adiposity rebound that usually occurs at around 5 years old [15].

In chapter 5, sex- and obesity- group specific means were estimated for the trajectory parameters, simultaneously adjusting for any potential birth cohort effect on the BMI trajectories by including centered "year of birth" (YOB) of each participant as a continuous level-2 covariate (i.e.YOB is centered around 1962, the year of birth of the oldest cohort in the sample). This strategy considers that the fixed growth parameters potentially vary linearly with year of birth, which is a simpler alternative than allowing for a completely different time trend in the response for each of the six birth cohorts [78].

The model used in this application can be written as follows:

Level-1 model: (1.1)

$$BMI_{ij} = b_{0i} + b_{1i}age_{ij}.(1 - u_{CP_i}(age_{ij})) + b_{2i}(age_{ij} - CP_i).u_{CP_i}(age_{ij}) + \varepsilon_{ij}$$

Level -2 model: (1.2)

$$b_{0i} = \beta_{0k} + \beta_{0yob} YOB_i + v_{0i}$$
$$b_{1i} = \beta_{1k} + \beta_{1yob} YOB_i + v_{1i}$$
$$b_{2i} = \beta_{2k} + \beta_{2yob} YOB_i + v_{2i}$$
$$CP_i = CP_k + \beta_{CPyob} YOB_i + v_{CPi}$$

And: (1.3)

$$\begin{pmatrix} v_{0i} \\ v_{1i} \\ v_{2i} \\ v_{CPi} \end{pmatrix} \sim N\left[\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma^2_{v0} & \dots & \cdots & \dots \\ \sigma_{v01} & \sigma^2_{v1} & \dots & \dots \\ \sigma_{v02} & \sigma_{v12} & \sigma^2_{v2} & \dots \\ \sigma_{v0CP} & \sigma_{v1CP} & \sigma_{v2CP} & \sigma^2_{CP} \end{pmatrix}\right]$$

Where BMI_{ij} is the BMI response of participant *i* at age *j*. $u_{CP_i}(age_{ij})$ is a unit heavyside step function where $u_{CP_i}(age_{ij})$=1 if $age_{ij} \geq CP_i$ and $u_{CP_i}(age_{ij}) = 0$, if $age_{ij} < CP_i$. Each participant *i* belong to a single group *k* (*k=1…8*) which identifies individual sex and obesity status group as in the Table 1 below.

Table 1. Eight sex by adiposity groups of participants considered in Chapter 5 analyses

K	Obesity status	Sex
1	Reference (normative)	Males
2	Reference (normative)	Females
3	Resolving obese	Males
4	Resolving obese	Females
5	Persistent obese	Males
6	Persistent obese	Females
7	Incident obese	Males
8	Incident obese	Females

$\beta_{0k}, \beta_{1k}, \beta_{2k}$ and CP_k in equation (1.2) are the expected trajectory parameters: β_{0k}, the intercept is the expected BMI at 25 years of age in group *k* given the participant is in the first phase of growth b_{1K}.; β_{1k}, the slope in childhood (S_1) which can be interpreted as the rate of change in BMI in the first phase of development; β_{2k} , the deviation between the childhood and adult slopes (S2 =adult rate - S_1) ; and CP, the change point for each sex and obesity status group *k*. Respectively, $\beta_{0yob}, \beta_{1yob}, \beta_{2yob}$ and β_{CPyob} are the expected linear increase in each trajectory parameters for each 1 year increase in year of birth (YOB).

ε_{ij} are the level 1 residual (i.e. random within-person error for person *i* at age *j* and are independent and normally distributed (i.e. $\varepsilon_{ij} \sim iid\ N(0, \sigma_e^2)$, and v_{0i}, v_{1i}, v_{2i} and v_{CPi} are the level 2 random deviations for person *i* from the group on initial BMI levels, rates of increase in BMI, and age at change point after controlling for group differences in the mean trajectory parameters. These random effects are multivariate normally distributed with zero mean and variances σ_{v0}^2 , σ_{v1}^2 , σ_{v2}^2 and σ_{CP}^2 respectively and full covariance matrix shown in 1.3. The variance-covariance matrix is not-group specific but estimated for the entire sample (N=2717 participants) since some of the *k* groups had less than 60 participants.

This parameterisation of the model with group-specific trajectory parameters is equivalent to the more traditional parameterisation that introduces group differences in the marginal growth parameters by considering group-specific deflections from population average for each growth-parameters [339] .

Note that the equations shown above are for a mode adjusted for a sex and obesity groups (i.e. 8 groups model). To decide whether potential sex heterogeneity needed to be accounted for in the trajectory model within obesity groups (i.e. whether there was a group and sex interaction on each growth parameters), three Bayesian hierarchical piecewise regression models were initially fitted with different level-2 sub models: one with adiposity group-specific growth parameters and no adjustment for sex (Model 1); one where growth parameters were allowed to vary by adiposity group with a main effect of sex (i.e. additive model 2); and one where all adiposity group-specific parameters were also allowed to vary by sex (Model 3 : sex by adiposity group interaction) (Table 2 below).

In concordance with data exploration, two commonly used Bayesian indices of model fit, the deviance information criteria (DIC) [306] and the Bayesian posterior predictive P-value (based on the sum of model residuals as a discrepancy measure [339]), indicated that the sex-specific model (Model 3) was a better fit than models that ignored potential developmental differences within obesity group between males and females, despite being more complex (see Table 2). Therefore, this more complex model was the one considered throughout the analyses in this chapter (see Chapter 4 for further details on model formulation and estimation).

Table 2. DIC and PP-values for the 3 considered parameterisation of the model.

Model	Level 2 Model	# parameters	DIC (pd)	Bayesian PP-val
1	~ Obesity group	16	19450 (2341)	0.45
2	~Obesity +sex	20	19467 (2235)	0.42
3	**~Obesity * sex**	**32**	**19003 (2790)**	**0.51**

One of the advantages of using MCMC techniques is that new samples of marginal posterior distributions of new variables can be derived from the chains obtained in the Gibbs sampling process. Thus, to compare parameters from two different growth curves (i.e. Group I males vs. Group I females), the marginal posterior density of the difference between parameters was thus obtained by calculating the difference between chains in each sample. If the density distribution (95% Credible interval) of each new variable of interest (i.e. difference in trajectory parameter between 2 groups) included zero, that there was no evidence to support the hypothesis that the two groups have significantly different parameters [125, 377].

Uninformative priors and hyper priors were used to fit the sex- and adiposity group-specific Bayesian hierarchical piecewise regression model. In this application, a restricted variance covariance structure was considered and specified with mutually independent random effects υ_{0i}, υ_{1i} , υ_{2i} and CP_i. This particular structure was used because the model is quite heavily parametrised and the primary focus is on the comparison of marginal means of growth

parameters between sex-and adiposity groups rather than the within-person correlations between different aspects of BMI change over time. All normal distributions were parameterised in terms of a mean and a precision (1/variance). β_{0k} , β_{1k} and β_{2k} for each sex and obesity group were normally distributed with a mean of 0 and a large variance. The population average change points estimated by log-likelihood profiling were used as priors for the change point means in each group as follows: CP_k*~N(16, 0.001)* for female sex-adiposity groups, and CP_k*~N(22,0.001))* for male sex-adiposity groups (see Chapter 4 for more details). All cohort effect priors (β_{0yob}, β_{1yob}, β_{2yob} and β_{CPyob}) were also given a flat normal prior (*N(16,0.001)*). The mutually independent random effects υ_{0i}, υ_{1i}, υ_{2i} and CP_i were normally distributed around 0 and a precision term *Tau* (τ =1/variance) that followed a gamma ($\tau \sim Gamma$ (0.01,0.01)). The variance of the error term was also given a gamma prior (*1/* $\sigma_e^2 \sim Gamma$ (0.01,0.01)). The BHPR model was ran using four parallel chains, and after a burn-in of 20000 iterations, each sequence was taken to 50000 iterations and thinned by 1/10 to reduce serial autocorrelation of the MC chains. The posterior distribution for the mean parameters (fixed effects) and the variance-covariance parameters (random effects) were obtained by mixing the four sequences. The convergence of the chains was assessed visually using traceplots and based on the Gelman-Rubin convergence criteria [124].

Note: Model convergence was not reached when we repeated the analyses using a "high-BMI resolving" group that considered strictly obese participants in childhood (which reduced the group to only 34 persons). It was therefore not possible to describe the growth of such a subsample with a four parameters model, and we only reported results for the same categories defined by Juoanala et al. in the previous study.

R code used to fit the models in Chapter 5:

```
##############################################################################
### SEX/OBESITY GROUP SPECIFIC MODEL : ALL GROUPS MODELLED SIMULTEANOULSY ###
##############################################################################
```

```
## BUGS model
bugs.model <- "
model {
## Likelihood
for(k in 1:N) {
y[k] ~ dnorm(mu[k],tau)
# formulation that works for the fixed effects :
#mu[k] <-
(b1[Grp[k],sex[k]]+re1[id[k]])+(b2[Grp[k],sex[k]]+re2[id[k]])*Age[k]+(b2[Grp[k],sex[k]]+
re3[id[k]])*step(Age[k]-CP[Grp[k],sex[k]]-re4[id[k]])*(Age[k]-CP[Grp[k],sex[k]]-
re4[id[k]])

# with obesity*sex interaction in the reference grid  ( 8 groups):one formulation that works
()
mu[k] <-
(b1[Grp[k]]+re1[id[k]])+(b2[Grp[k]]+re2[id[k]])*Age[k]+(b3[Grp[k]]+re3[id[k]])*step(Age
[k]-CP[Grp[k]]-re4[id[k]])*(Age[k]-CP[Grp[k]]-re4[id[k]])
#mu[k] <- b[1]+re1[id[k]]+(b[2]+re2[id[k]])*Age[k]+(b[3]+re3[id[k]])*step(Age[k]-
(CP+betaGroup*(Grp[k]-1))-re4[id[k]])*(Age[k]-CP-betaGroup*(Grp[k]-1)-re4[id[k]])

# likelihood FORMULATION with cohort effect :
#mu[k] <-
(b1[Grp[k]]+re1[id[k]]+b1yob*YOB[k])+(b2[Grp[k]]+re2[id[k]]+b2yob*YOB[k])*Age[k]+
(b3[Grp[k]]+re3[id[k]]+b3yob*YOB[k])*step(Age[k]-CP[Grp[k]]-re4[id[k]]-
CPyob*YOB[k])*(Age[k]-CP[Grp[k]]-re4[id[k]]-CPyob*YOB[k])
# or is it more like :
#mu[k] <-
(b1[Grp[k]]+re1[id[k]]+b1yob*YOB[id[k]])+(b2[Grp[k]]+re2[id[k]]+b2yob*YOB[id[k]])*
Age[k]+(b3[Grp[k]]+re3[id[k]]+b3yob*YOB[id[k]])*step(Age[k]-CP[Grp[k]]-re4[id[k]]-
CPyob*YOB[id[k]])*(Age[k]-CP[Grp[k]]-re4[id[k]]-CPyob*YOB[id[k]])
```

```
}

##independent  Random Effects : idem
for(k in 1:M) {
re1[k] ~ dnorm(0,tau1)
re2[k] ~ dnorm(0,tau2)
re3[k] ~ dnorm(0,tau3)
re4[k] ~ dnorm(0,tau4)
}


## Priors - precision for independet random effects
tau ~ dgamma(0.001,0.001)
tau1 ~ dgamma(0.001,0.001)
tau2 ~ dgamma(0.001,0.001)
tau3 ~ dgamma(0.001,0.001)
tau4 ~ dgamma(0.001,0.001)

## Mean is zero here because we subtract 25 from Age
#for(g in 1:G) { #G = nb groups
#for(s in 1:2){ # two sexes
#CP[g,s] ~ dnorm(0,0.0001)
#}
#}


#for(g in 1:G) { #G = nb groups
#for(s in 1:2){ # two sexes
#b1[g,s] ~ dnorm(25,0.0001)
#}
```

```
#}

#for(g in 1:G) { #G = nb groups
#for(s in 1:2){ # two sexes
##b2[g,s] ~ dnorm(0,0.0001)
#}
#}

#for(g in 1:G) { #G = nb groups
#for(s in 1:2){ # two sexes
#b3[g,s] ~ dnorm(0,0.0001)
#}
#}

# WITH INTERACTION IN REF GRID:
#for(i in 1:8){
#b1[i] ~ dnorm(25,0.0001)
#b2[i] ~ dnorm(0,0.0001)
#b3[i] ~ dnorm(0,0.0001)
#CP[i] ~ dnorm(0,0.0001)
#}

# more informative
b1[1] ~ dnorm(28,0.0001)
b1[2] ~ dnorm(28,0.0001)
b1[3] ~ dnorm(31,0.0001)
b1[4] ~ dnorm(32,0.0001)
b1[5] ~ dnorm(28,0.0001)
b1[6] ~ dnorm(27,0.0001)
b1[7] ~ dnorm(24,0.0001)
b1[8] ~ dnorm(24,0.0001)
```

b2[1] ~ dnorm(0.5,0.0001)
b2[2] ~ dnorm(0.5,0.0001)
b2[3] ~ dnorm(0.52,0.0001)
b2[4] ~ dnorm(0.54,0.0001)
b2[5] ~ dnorm(0.6,0.0001)
b2[6] ~ dnorm(0.6,0.0001)
b2[7] ~ dnorm(0.45,0.0001)
b2[8] ~ dnorm(0.48,0.0001)

b3[1] ~ dnorm(-0.5,0.0001)
b3[2] ~ dnorm(-0.5,0.0001)
b3[3] ~ dnorm(-0.3,0.0001)
b3[4] ~ dnorm(-0.2,0.0001)
b3[5] ~ dnorm(-0.6,0.0001)
b3[6] ~ dnorm(-0.5,0.0001)
b3[7] ~ dnorm(-0.39,0.0001)
b3[8] ~ dnorm(-0.38,0.0001)

CP[1] ~ dnorm(-2.6,0.0001)
CP[2] ~ dnorm(-9,0.0001)
CP[3] ~ dnorm(0,0.0001)
CP[4] ~ dnorm(4,0.0001)
CP[5] ~ dnorm(5,0.0001)
CP[6] ~ dnorm(5,0.0001)
CP[7] ~ dnorm(-0.3,0.0001)
CP[8] ~ dnorm(-7,0.0001)

PRIORS FOR cohort effects :
b1yob ~ dnorm(0,0.0001)
b2yob ~ dnorm(0,0.0001)

```
b3yob ~ dnorm(0,0.0001)
CPyob ~ dnorm(0,0.0001)

## Transform parameters
# intercepts :
# resolving
#b1gp0males <-b1[1,1]
#b1gp0females <-b1[1,2]

# persisent
#b1gp1males <-b1[2,1]
#b1gp1females <-b1[2,2]
# differences :
#b1diffmales <-b1gp0males-b1gp1males
#b1difffemales <-b1gp0females-b1gp1females

# incidend
#b1gp2males <-b1[3,1]
#b1gp2females <-b1[3,2]

# reference :
#b1gp3males <-b1[4,1]
#b1gp3females <-b1[4,2]

sigma <- 1/sqrt(tau)
sigma1 <- 1/sqrt(tau1)
sigma2 <- 1/sqrt(tau2)
sigma3 <- 1/sqrt(tau3)
sigma4 <- 1/sqrt(tau4)

## Predictions
```

```
re1.pr ~ dnorm(0,tau1)
re2.pr ~ dnorm(0,tau2)
re3.pr ~ dnorm(0,tau3)
re4.pr ~ dnorm(0,tau4)

# retrieve random effects?
#for(k in 1:M) {
#re1<-re1[k]
#}
#RE1<-re1

# RETRIEVE AVERAGE CURVES FOR EACH OF THE 8 GROUPS :

for(k in 1:P){
y1[k] <- b1[1]+(b2[1])*Age.pr[k]+(b3[1])*step(Age.pr[k]-CP[1])*(Age.pr[k]-CP[1])
y2[k] <- b1[2]+(b2[2])*Age.pr[k]+(b3[2])*step(Age.pr[k]-CP[2])*(Age.pr[k]-CP[2])
y3[k] <- b1[3]+(b2[3])*Age.pr[k]+(b3[3])*step(Age.pr[k]-CP[3])*(Age.pr[k]-CP[3])
y4[k] <- b1[1]+(b2[4])*Age.pr[k]+(b3[4])*step(Age.pr[k]-CP[4])*(Age.pr[k]-CP[4])
y5[k] <- b1[5]+(b2[5])*Age.pr[k]+(b3[5])*step(Age.pr[k]-CP[5])*(Age.pr[k]-CP[5])
y6[k] <- b1[6]+(b2[6])*Age.pr[k]+(b3[6])*step(Age.pr[k]-CP[6])*(Age.pr[k]-CP[6])
y7[k] <- b1[7]+(b2[7])*Age.pr[k]+(b3[7])*step(Age.pr[k]-CP[7])*(Age.pr[k]-CP[7])
y8[k] <- b1[8]+(b2[8])*Age.pr[k]+(b3[8])*step(Age.pr[k]-CP[8])*(Age.pr[k]-CP[8])
}

########################################
## SAMPLE BETWEEN-GROUP DIFFERENCES :
########################################

###### INTERCEPT ########
###RESOLVE/PERSIST :
b1FMres<-b1[1]-b1[2] #males -females
```

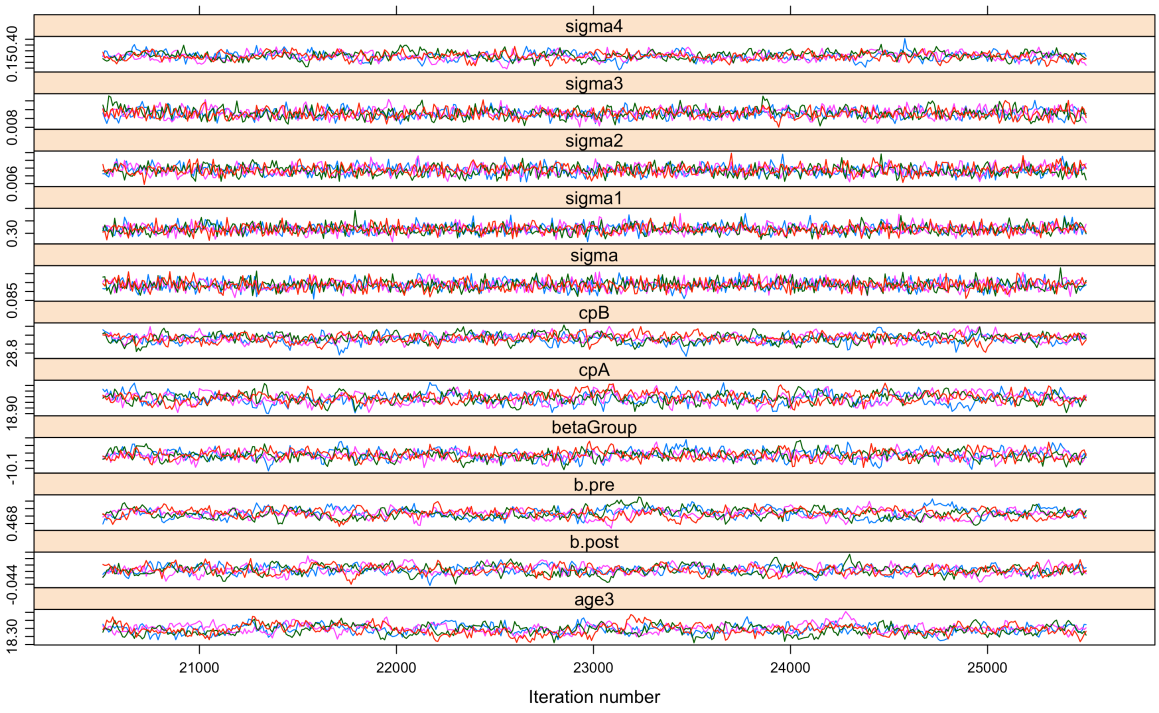
```
b1FMpersis<-b1[3]-b1[4]
b1MpersisResolv<-b1[1]-b1[3]#resolv-persis
b1FpersisResolv<-b1[2]-b1[4]
###REFERENCE/INCIDENT :
b1FMinc<-b1[5]-b1[6] #males -females incident
b1FMref<-b1[7]-b1[8]
b1Mincref<-b1[5]-b1[7]# male incident-male ref
b1Fincref<-b1[6]-b1[8]# female incident-female ref

###### childhood slope  ########
###RESOLVE/PERSIST :
b2FMres<-b2[1]-b2[2] #males -females
b2FMpersis<-b2[3]-b2[4]
b2MpersisResolv<-b2[1]-b2[3]#resolv-persis
b2FpersisResolv<-b2[2]-b2[4]
###REFERENCE/INCIDENT :
b2FMinc<-b2[5]-b2[6] #males -females incident
b2FMref<-b2[7]-b2[8]
b2Mincref<-b2[5]-b2[7]# male incident-male ref
b2Fincref<-b2[6]-b2[8]# female incident-female ref

###### adult slope ########
###RESOLVE/PERSIST :
b3FMres<-b3[1]-b3[2] #males -females
b3FMpersis<-b3[3]-b3[4]
b3MpersisResolv<-b3[1]-b3[3]#resolv-persis
b3FpersisResolv<-b3[2]-b3[4]
###REFERENCE/INCIDENT :
b3FMinc<-b3[5]-b3[6] #males -females incident
b3FMref<-b3[7]-b3[8]
b3Mincref<-b3[5]-b3[7]# male incident-male ref
```

```
b3Fincref<-b3[6]-b3[8]# female incident-female ref

###### changepoint #########
###RESOLVE/PERSIST :
CPFMres<-CP[1]-CP[2] #males -females
CPFMpersis<-CP[3]-CP[4]
CPMpersisResolv<-CP[1]-CP[3]#resolv-persis
CPFpersisResolv<-CP[2]-CP[4]
###REFERENCE/INCIDENT :
CPFMinc<-CP[5]-CP[6] #males -females incident
CPFMref<-CP[7]-CP[8]
CPMincref<-CP[5]-CP[7]# male incident-male ref
CPFincref<-CP[6]-CP[8]# female incident-female ref



}
"
## Initialize JAGS
library(rjags)

# creat interaction term to get 8 groups base on sex and obseity status :
BMIdata$group =interaction(BMIdata$sex,BMIdata$obesity) # 8 possible Levels: 1.0 2.0
1.1 2.1 1.2 2.2 1.3 2.3
inits=list(b1=c(28,25,30,30,28,28,22,23),b2=c(0.5,0.5,0.5,0.5,0.5,0.5,0.5,0.5),b3=c(-0.45,-
0.45,-0.1,0,-0.35,-0.35,-0.35,-0.35),CP=c(-3,-9,2,10,2,2,-3,-9),tau=100)

Age.pr <- c(6,10,12, 15,17,20,25,30,40,50) # AGES FOR PREDICTION
BMIdata=subset(BMIdata,subset=!is.na(obesity))
model <- jags.model(textConnection(bugs.model),
                    data = list("y" = BMIdata$bmi,
                                "Age" = as.numeric(BMIdata$age)-25,
```

```
                    "YOB"= as.numeric(BMIdata$YOB)-69,
                    "Age.pr" = Age.pr-25,
                    "id" =  as.integer(as.factor(BMIdata$id)),#
                    "G"=as.integer(nlevels(as.factor(BMIdata$obesity))),# 4 obesity gp
                    "Grp" = as.integer(BMIdata$group), # 1,2,3,4 to 8
                    "N" = as.integer(nrow(BMIdata)),# rows
                    "M" = as.integer(nlevels(as.factor(BMIdata$id))),#3546
                    "P" = length(Age.pr)),
              inits=inits,
              n.chains = 3,
              n.adapt = 5000)


update(model,1000)
# MCMC SAMPLING:
# what do we retrieve?
s <- coda.samples(model,

var=c("b1","b2","CP","b3","sigma","sigma1","sigma2","sigma3","sigma4","b1FMres","b1F
Mpersis","b1MpersisResolv","b1FpersisResolv","b1FMinc","b1FMref","b1Mincref","b1Fin
cref","b2FMres","b2FMpersis","b2MpersisResolv","b2FpersisResolv","b2FMinc","b2FMre
f","b2Mincref","b2Fincref","b3FMres","b3FMpersis","b3MpersisResolv","b3FpersisResolv
","b3FMinc","b3FMref","b3Mincref","b3Fincref",

"CPFMres","CPFMpersis","CPMpersisResolv","CPFpersisResolv","CPFMinc","CPFMref",
"CPMincref","CPFincref"),
              n.iter=30000,thin=20)
# sample cohort effects:
scohort <- coda.samples(model,
              var=c("b1yob","b2yob","b3yob","CPyob"),
              n.iter=10000,thin=10)
```

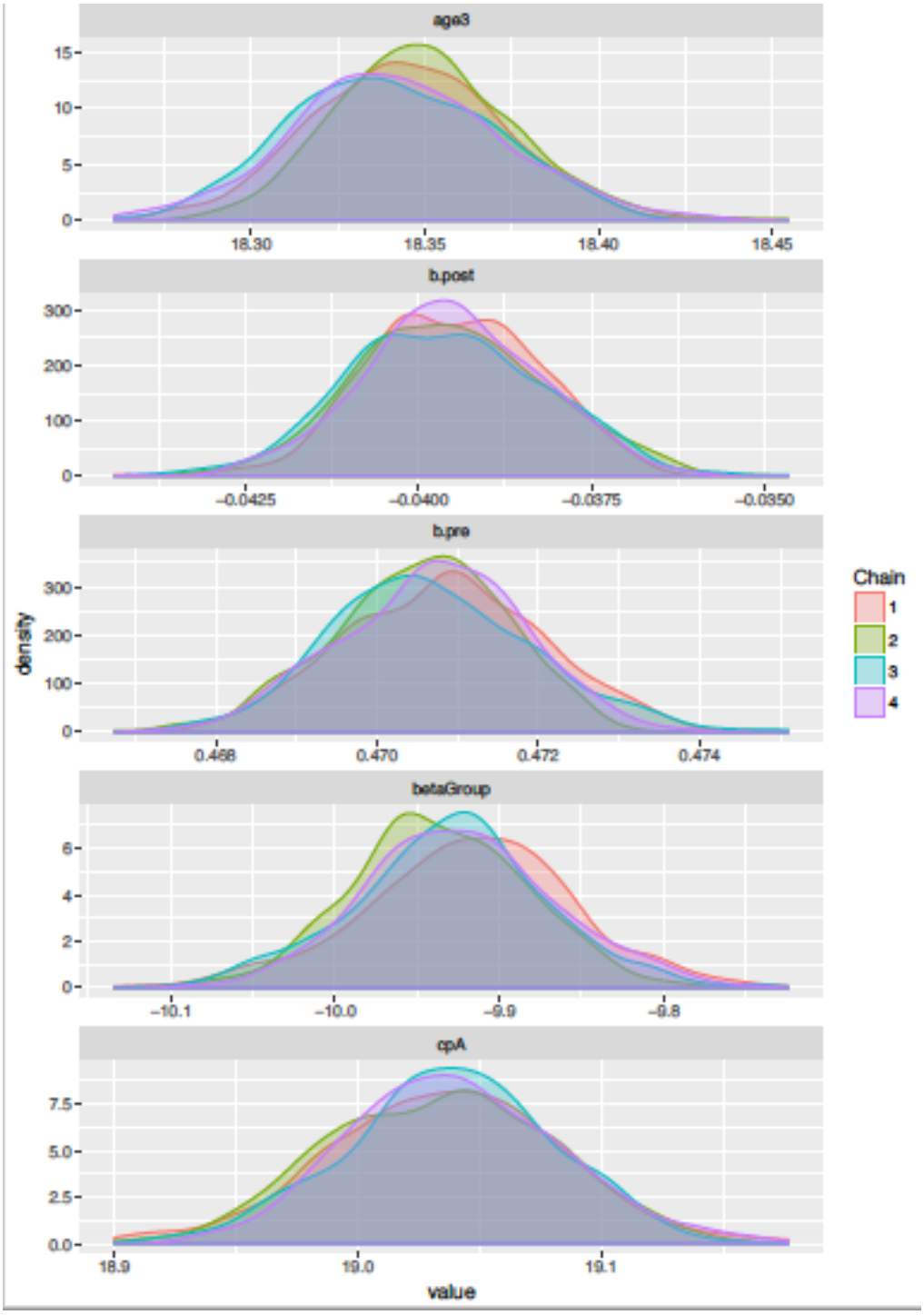
```
# smaple predictions
sy <- coda.samples(model,
                   var=c("y1","y2","y3","y4","y5","y6","y7","y8"),n.iter=30000,thin=20)
#write.csv(summary(s), file = "summary.csv") ;NOT WORKING

#"b1FMres","b1FMpersis","b1MpersisResolv","b1FpersisResolv","b1FMinc","b1FMref","b
1Mincref","b1FpersisResolv"
levels(BMIdata$group) #"1.0" "2.0" "1.1" "2.1" "1.2" "2.2" "1.3" "2.3"
summary(s)# actual parameters
plot(s[,"b1[8]"])
summary(sy)# average trajectories for each of the 8 group

# Predicted values:
summary(sy)$stat[,1]# all values (10 for each group)



# create prediction data.frame
pred<-data.frame(group=rep(c("1.0", "2.0", "1.1", "2.1" ,"1.2", "2.2" ,"1.3"
,"2.3"),each=length(Age.pr)),
                 Age=rep(Age.pr,8),
                 y=summary(sy)$stat[,1])
```

Appendix 5.9. Model Validation traceplots for the best fitting model used to compute BMI trajectory parameters for the 4 adiposity groups for males and females in Chapter 5.

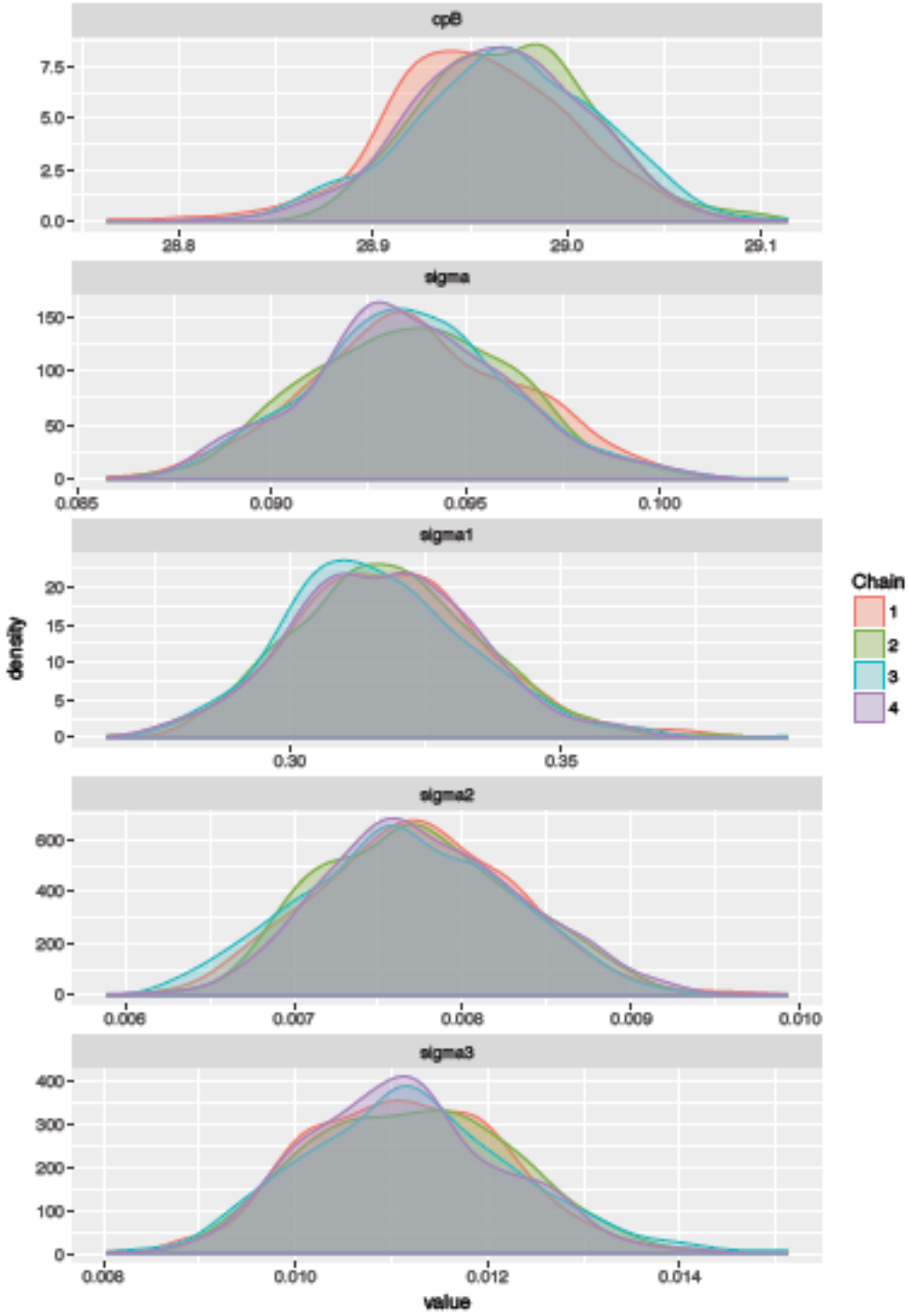


Appendix 5.10. Other model validation plots and Bayesian model-fitting diagnostic statistics for the best fitting model used to compute BMI trajectory parameters for the 4 adiposity groups for males and females in Chapter 5.

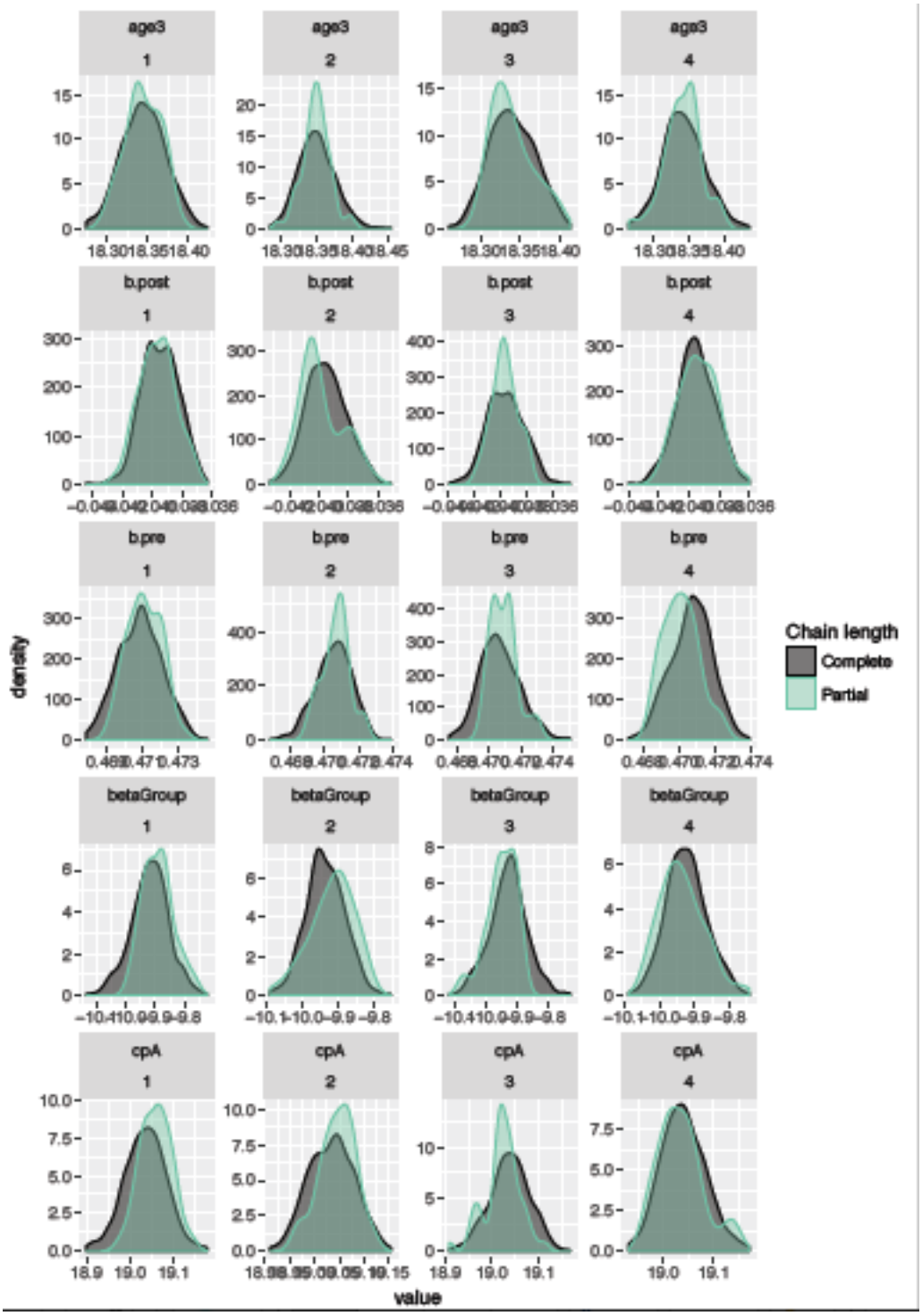
Posterior density plots of parameters:



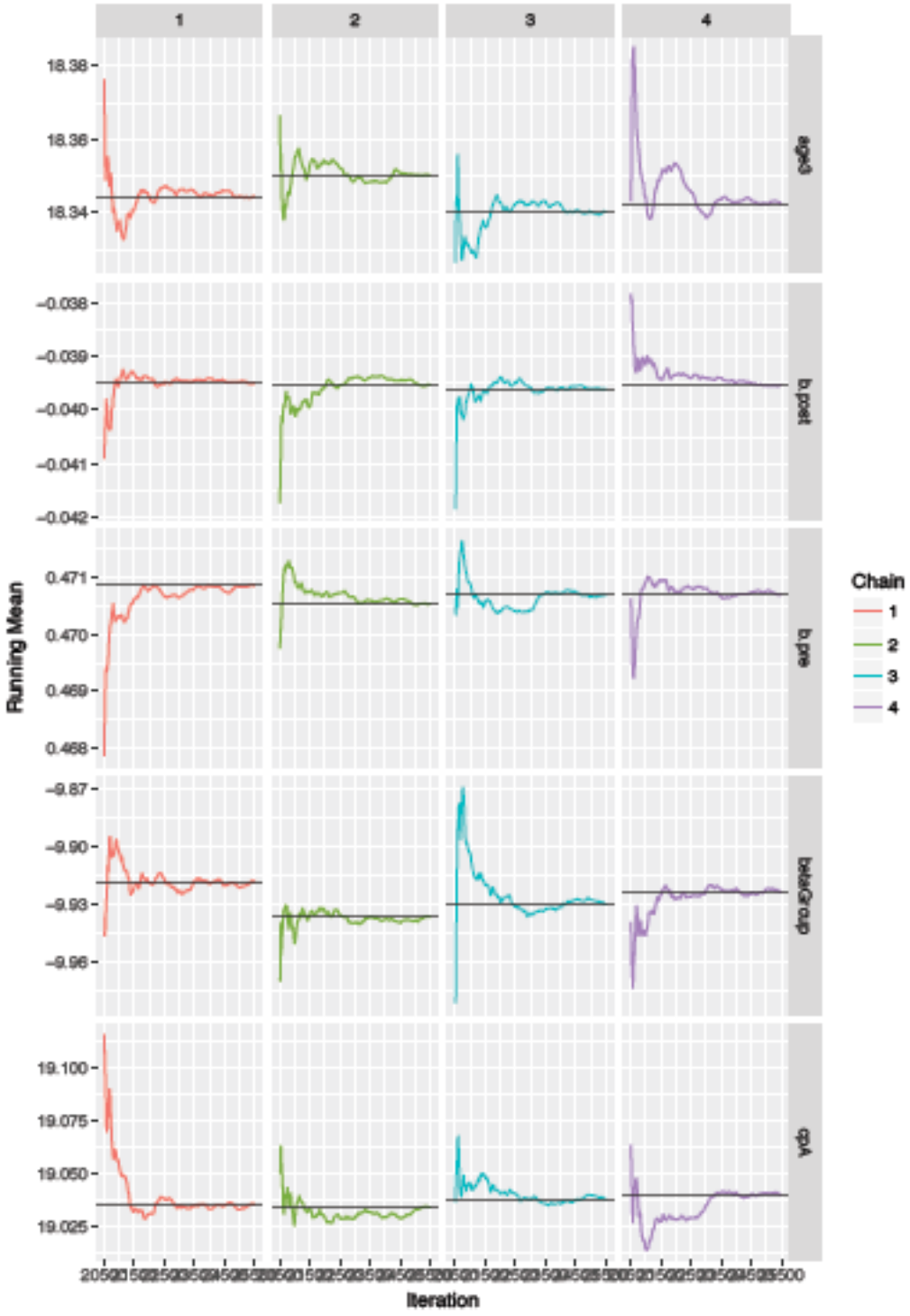
Posterior density plots of parameters: (continued):



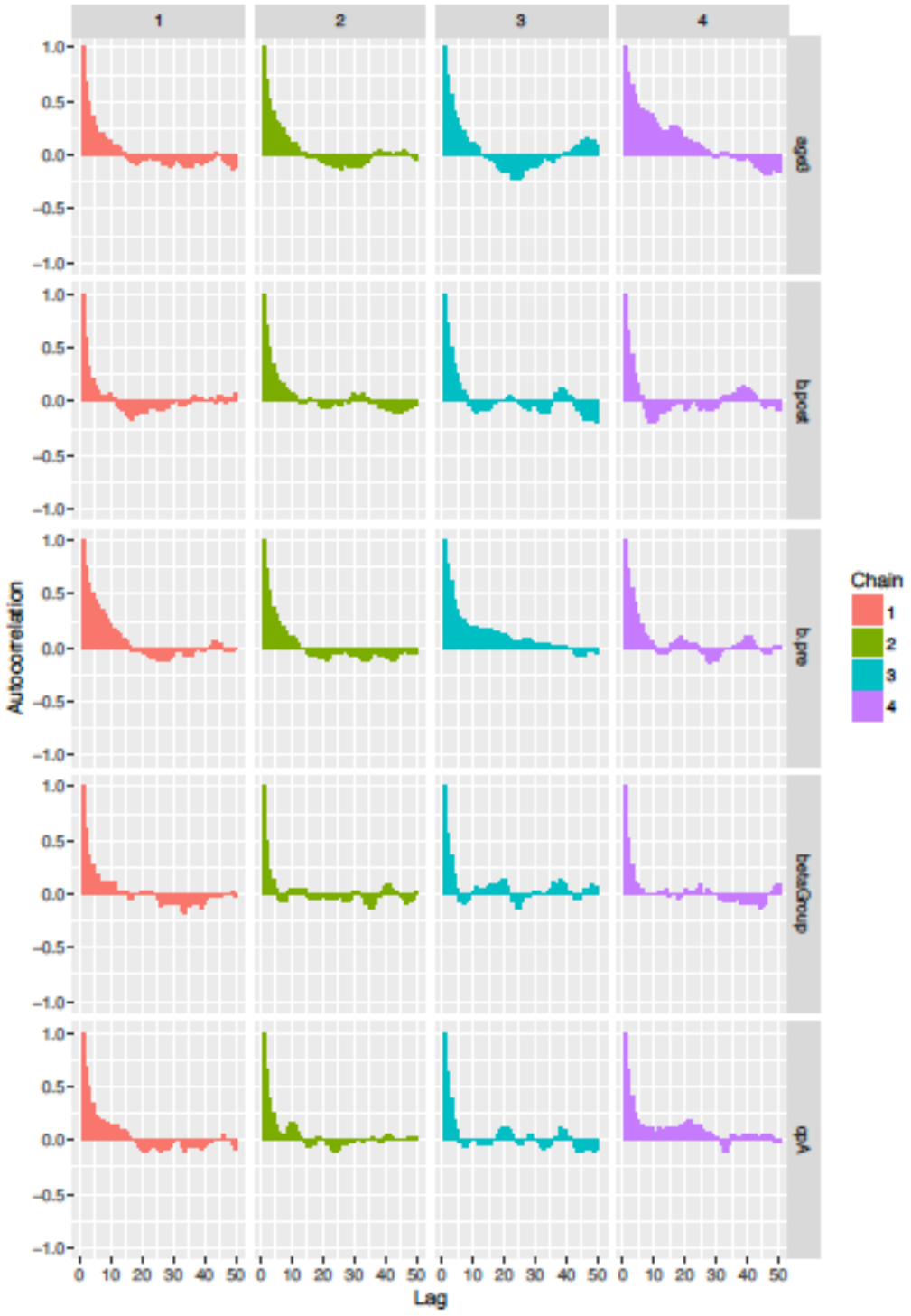
Comparison of partial and full chain:



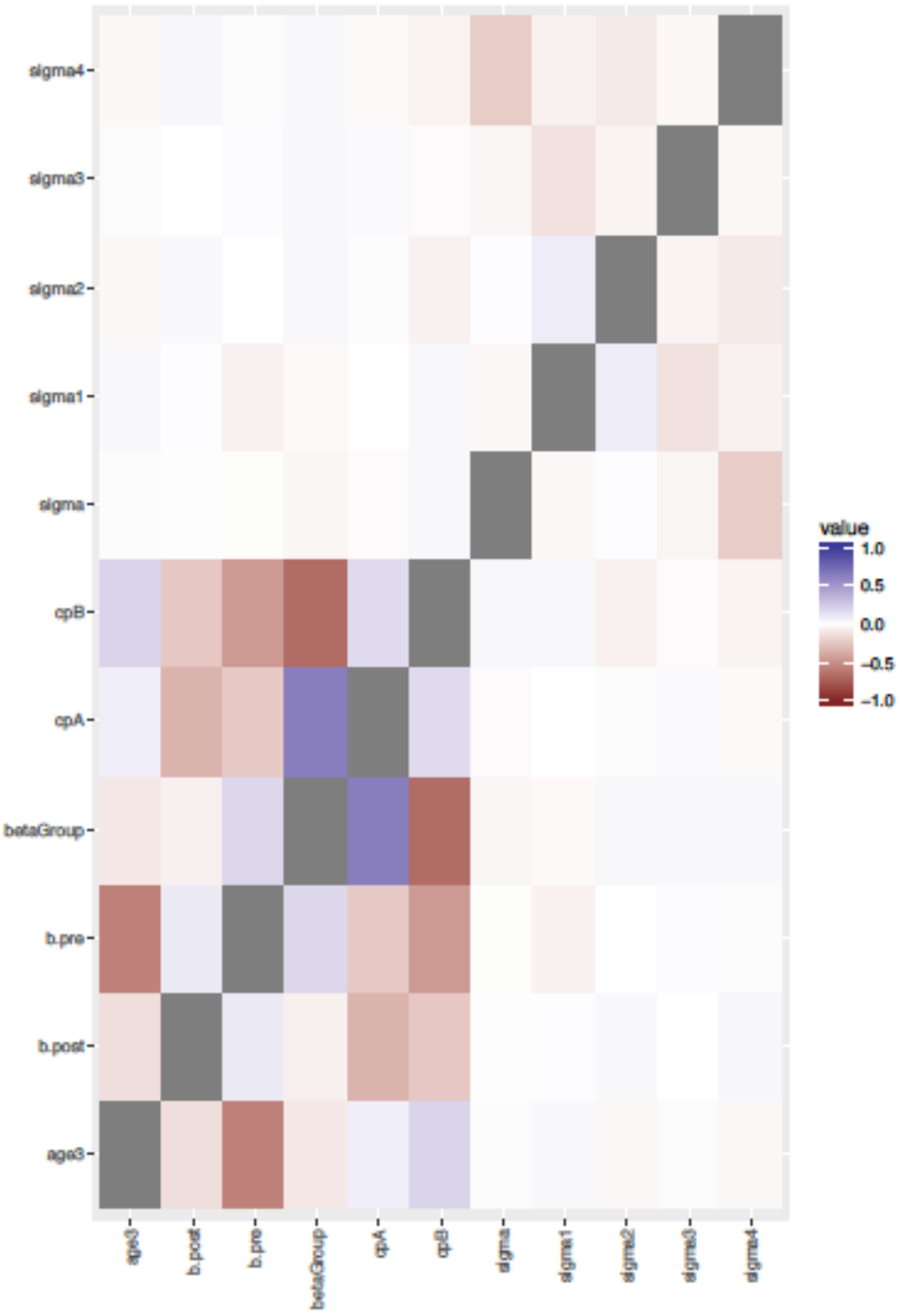
Running means:



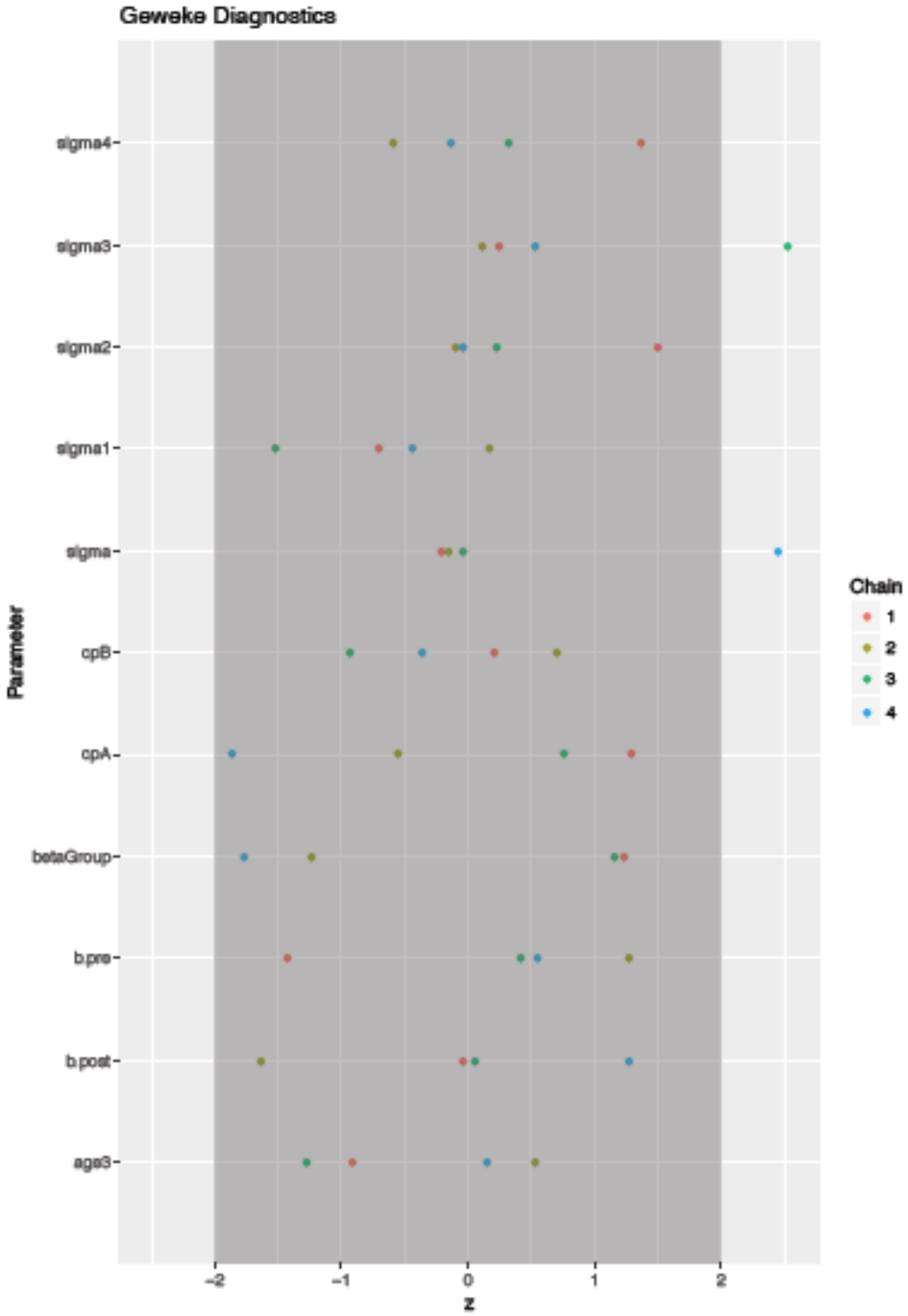
Autocorrelation plot:



Crosscorrelation plot:



Geweke z-score diagnostic plot:



Appendix 6.1. Growth Mixture modeling for identifying latent Class BMI trajectories

The estimation of latent class models were performed with the `lcmm` package in `R`, which regroups a series of functions to estimate latent class mixed models for Gaussian and non-Gaussian longitudinal outcomes [361]. As recommended in the literature [220, 225, 226, 231, 362], various LGCMM models were fit and compared before choosing a final model. Participants' ages in the analyses were centrered around 25 years, the mean age in the population sample, to facilitate the interpretation of the intercepts and improve numerical stability. Three possible polynomial specifications were considered to describe the within-person shape of the longitudinal BMI response as a function of age: a linear, and also a quadratic and a cubic specification, which allowed for curvilinear developmental patterns of BMI. Each of these polynomial models (order 1 to 3) were respectively modeled as a one to seven class solution, starting with a standard one-class heterogeneous mixed model where all subjects are assumed to follow the same underlying trajectory over time, and increasing the number of latent classes progressively.

For each of these models the specification of the intercept and slope(s) variance (i.e. random effects) was considered to allow for between-person heterogeneity in the BMI response. To limit the number of potential models tested, the variances of random intercept and slopes were constrained to be equal across classes, and the systematic approach of Feldman et al [363] was used to relax the constraints on the variance/covariance matrix. The linear, quadratic, and cubic growth mixture models were tested respectively with intercept variance only and with intercept and slopes variances. When relevant, each model was tested with all possible covariances estimated, and with all covariances constrained to zero. In general, the more estimated covariances; the fewer classes could be successfully extracted from the models, that is models with large number of classes failing to converged (i.e. singular matrix) or were unable to generate standard error of parameters. However, all classes could successfully be estimated when the covariances of all random effects were constrained to zero for all models (data not shown). All models presented in Appendix 6.2 were defined with a block diagonal structure for the random intercept and slopes, which represent varying but uncorrelated growth parameters.

As recommended by Proust-Lima et al. [361], models with two or more classes were ran several times with different sets of initial values to avoid convergence towards local maxima, a problem commonly encountered when fitting mixture models. Each model was fit with (1) initial values generated from the maximum likelihood estimates of the corresponding 1–class model, (2) initial values randomly generated from the asymptotic distribution of the corresponding 1-class model, and (3) initial values obtained via grid searching (with a maximum of 20 iterations from 30 random vectors of values from the 1-class model)[361]. The results of the series of models that were fitted solely to ensure the correct convergence of the program algorithm towards global maximum of the log-likelihood are not shown. The models results presented in chapter 6 are those of growth mixture models that showed satisfactory evidence of convergence.

For each model involving latent classes, posterior class-membership probabilities were used to obtain a posterior classification of the participants in each latent class to evaluate goodness-of-fit and to characterise the discrimination of latent clusters (See `lcmm` documentation for details on post-fit computations). The proportion of subjects classified in each class with a posterior probability above a threshold of 0.7 was also retrieved and indicates the proportion of subjects unambiguously classified in each latent class. Thirdly, the mean of posterior probabilities of belonging to the latent class among subjects classified a posteriori in each class was recorded (i.e. mean probabilities between 0.8 and 1 indicate a good classification). Model fit was further assessed by examination of model residuals and plots of weighted mean predictions of each model. As recommended in the literature, the choice of the best fitting model was based on BIC values, and on these other index of goodness of fit and discrimination [361]. When comparing different LCGMM models, it is recommended that to be considered a significant improvement over an existing model, a reduction of BIC of at least 10 points is necessary in the competing model [229].The best fitting model in terms of BIC were the 7 class quadratic and cubic models, but these models had poor discrimination for some classes (mean posterior probabilities <0.65 for several classes, less than 60 % of participants classified in several classes with a posterior

probability above 0.7). Furthermore, when seven classes were considered, some classes contained less than 1% of participants. In contrast, the six class quadratic model had only a slightly larger BIC, a higher mean posterior probability in all six classes (> 0.78), and a high proportion of participants were classified in each of the six classes above the 0.7 threshold (70% of participants or above in each class). The parameter estimates of the fixed and random components of the six class quadratic mixture model are given in Appendix 6.3. The necessity of random intercept and slope variance within-class was assessed for this final model, but the results of these further analyses are not shown since these additional models did not result in a sufficient increase in BIC to justify class-specific variance components.

Note that when fitting the suite of LCGMM models on the entire subset of 2717 participants, including the 86 participant who only had 2 BMI records (one at baseline and one in adulthood) resulted in largely identical latent trajectories, but the mean posterior probabilities in each class were slightly worse, as expected, since it is difficult to assign these participants to one of the six identified trajectories when they only have two observations (see table 3. below).

Table 3. Best fitting LCGMM model restuls for the sample that included 86 participants with only two BMI records

6 classes-quadratic model	**Nb. Participants in each class**	**Mean posterior Probabilities**	**Posterior probabilities > 0.7 (%)**
N= 2717	1495 /40 /902/ 129 /115/36	0.88 /0.76 /0.80 /0.79 / 0.80 /0.92	86.1/ 62.5/ 73.5/ 65.2 /66.1/ 91.61

Appendix 6.2. Latent Class Growth Mixture models (LCGMM) results.

Nb. Latent classes	Polynomial degree	Log-Lik	BIC	% Participants per class	Mean posterior probabilities	Posterior probabilities > 0.7 (%)
	Linear	-33513.66	67066.86	100	n.a	n.a
1	Quadratic	-32599.90	65259.99	100	n.a	n.a
	Cubic	-32597.43	65266.02	100	n.a	n.a
	Linear	-32793.43	65650.12	86.8 /13. 2	0.97 / 0.91	97.33 / 87.43
2	Quadratic	-31859.42	63805.81	86.3 /13.7	0.98 / 0.92	97.92 / 88.12
	Cubic	-32597.43	63829.9	85.2 /14. 8	0.97 / 0.88	97.2 / 82.43
3	Linear	-32565.23	65217.45	74.1 / 22.6 /3.2	0.93 / 0.85 /0.93	92.4 / 81.9/ 91.0
	Quadratic	-31608.36	63335.33	71.4 / 24.8 /3.7	0.93 / 0.85 /0.94	92.3 / 85.7 / 94
	Cubic	-31754.48	63359.19	70.6 / 25.4 / 4.0	0.92 / 0.84 /0.93	91.6 / 8.5 / 91.7
4	Linear	-32474.81	65060.32	55.8 / 33.2 / 9.2 /1.7	0.88 /0.82 / 0.85/ 0.93	85.3/ 69.0 / 81/ 84.4
	Quadratic	31467.54	63085. 2	62.5 /4.8 / 29.7/ 3.0	0.91 / 0.84 /0.81/0.90	89.7 / 80.00 / 73.9 / 81.7
	Cubic	-31461.75	63083.27	2.6 / 61.2 / 31.2 / 4.9	0.85 /0.90/ 0.83 /0.87	72.2/ 87.9/ 75.8/ 85.0
5	Linear	-32390.99	64916.41	1.9 / 35.0 / 9.1 / 52.3 / 1.7	0.81 /0.80/ 0.86/ 0.88 / 0.94	70.3 / 73.0 / 83.1/ 85.8 / 95.7
	Quadratic	-31280.68	62774.86	55.0/ 1.5/ 33.2 / 4.8 / 4.2 /1.3	0.88/ 0.78/ 0.82 / 0.82 /0.94	86.1/ 62.5 /73.5/ 66.1/ 91.6
	Cubic	-31278.64	62786.6	53.8 / 34.4 / 4.6 / 1.4 / 5.9	0.88/ 0.80/ 0.82 / 0.96 /0.83	85.7 / 72.5 /75.8 /94.7 /67.9
6	Linear	-32363.13	64884.41	48.1 / 9.9 / 2.1/ 2.3/ 36.5/ 1.1	0.86/ 0.80/ 0.81 / 0.84 /0.78 /0.90	83.9/ 72.2 / 69.7 / 82.3 / 70.9 / 87.1
	Quadratic	**-31280.68**	**62774.86**	**55.0 / 1.5 / 33.2 / 4.7/ 4.3/ 1.3**	**0.89 /0.78 / 0.81 / 0.80 /0.80 / 0.93**	**86.2 / 70.5 /73.50 /74.1 /71.1 / 92.7**
	Cubic	-31272.73	62779.31	52.3 / 3.7 /34.8 / 4.0 / 3.7 / 1.7	0.89 / 0.77 /0.8 / 0.77 / 0.8 / 0.94	85.8 / 61.4/ 72.2/ 60.00 / 69 /94.59
7	Linear	-32358.8	64899.47	42.6/ 35.6/ 9.4/ 7.3/ 2.1/ 1.9/ 0.9	0.83 /0.68/0.59 /0.74 /0.81/0.79 /0.89	78.0 /49.7/ 14.8/ 61.6/ 68.9/ 73.5/ 80
	Quadratic	-31245.25	62765.62	56.4/18.1/ 0.77/14.2 /4.7/ 4.4/1.3	0.76 /0.65/0.59 /0.61 /0.74/0.80 /0.81	82.1/ 37.1/ 66.7 /30.6 / 55.8/ 68.1/ 91.7
	Cubic	-31118.31	62555.01	33.4/ 4.6/ 51.2/ 1.0/ 4.0 / 4.7 /1.1	0.78 /0.75/0.58 /0.77 /0.75/0.57 /0.86	69.5 / 57.3/ 85.7/ 85.7/ 59.3/ 54.5/ 96.7

Reported are the number of latent class considered, the polynomial form of the model, the maximum Log-Likelihood (Log-Lik), the Bayesian information Criterion (BIC), and for models with two or more classes, the a posteriori classification of participants in each class (%), the mean of posterior probabilities in each latent class, and the % of participants classified in each class with a posterior probability above 0.7. The best fitting model is highlighted in bold characters. (n.a, not applicable).

Appendix 6.3. Parameter estimates for the best fitting six-class quadratic Latent Class Growth Mixture model fitted to the BMI data of 2631 YFS participants.

Fixed effects			
Latent Class	**Intercept (se)***	**Linear slope** (se)**	**Quadratic slope (se) ****
1	21.40 (0.07)	0.20 (3.10^{-2})	-0.005 (1.10^{-3})
2	30.17 (0.52)	0.19 (0.02)	-0.02 (2.10^{-2})
3	23.5 (0.13)	0.35 (5.10^{-2})	-0.006 (3.10^{-3})
4	27.11(0.25):	0.61 (0.01)	0.001(8.10^{-3})^{N}
5	30.87 (0.33)	0.49 (0.01)	-0.01 (9.10^{-3})
6	36.89 (0.41)	0.77 (0.01)	-0.01 (1.10^{-2})
Random effects: variance-covariance matrix			
σ^2_{int}= 2.56			
$\sigma^2_{linear\ slope}$= 0.004			
$\sigma^2_{quadratic\ slope}$= 0.001			
σ^2_{error} 1.39 (0.01)			

* Intercept interpreted as the expected level BMI at 25 years of age in Kg/m^2 (due to centering of age around mean age in the sample). All reported slopes are significant (Wald test, p-value <0.05), unless indicated by the "^{N}" subscript.

**Slopes in kg/m^2/year reported as: linear (se) /quadratic (se)/ cubic (se), when applicable. All reported slopes are significant (Wald test statistic with normal approximation, p-value <0.05), unless indicated by the "^{N}" subscript.

Appendix 6.4. Prevalence of adult overweight and obesity in the YFS subset assessed for BMI in 2001, 2007, and 2011.

Year*	Sex	Nb. Participants	% Overweight	% Obese
2001	M	999	38.9	11.47
	F	1237	24.78	13.73
	Total	2236	31.2	12.4
2007	M	962	45.9	18.7
	F	1172	28.4	16.2
	Total	2134	34.6	17.8
2011	M	912	43.4	0.2127
	F	1103	33.0	20.5
	Total	2015	36	20.85

*mean age (sd) of participants seen in 2001, 2007, and 2011 were 31.7 (4.8), 37.7 (5), and 41.9 (6) years, respectively.

Appendix 6.5. Prevalence of each cardiometabolic risk outcome in adulthood in the YFS cohort.

Adult cardiometabolic risk outcome	% of original cohort	Prevalence in assessed adults (%)*	Nb. missing adult risk assessment (%)**
Pre-atherosclerosis	8.6	11.6	944 (26.2)
High-risk triglycerides	8.5	11.1	826 (22.9)
High-risk HDL-C	15.1	19.6	826 (22.9)
High-risk LDL-C	12.2	14.8	644 (17.9)
Hypertension	16.4	19.1	644 (17.9)
Diabetes	2.5	3.5	1030(28.6)

* % of each adult cardiometolic outcome assessed in 2001, 2007, or 2011 (latest existing adult assessment)

** % Participants form the original cohort missing cardioemtaobolic outcome in adulthood

Appendix 6.6. Cumulative adult cardiometabolic risk load in adulthood.

		Adult CVD risk score					
		0	**1**	**2**	**3**	**4**	**5**
Latent BMI Trajectory class**	**1**	930	250	144	29	7	2
	2	22	13	5	1	0	1
	3	347	222	136	56	31	5
	4	27	29	21	11	8	0
	5	29	18	26	18	5	2
	6	5	8	9	3	3	3
Nb participants		1360	540	340	117	55	9
%*		56.1	22.4	14.1	4.9	2.5	0.4

The cumulative CVD risk load ranges from 0 to 6, depending on the number of deleterious CVD risk factors for each participant). (Mean score: 0.7588 (sd=1.05)).
*% Nb. participants for each score category / Number of participants with non-missing CVD risk scores (N=2421).
**Latent BMI trajectory classes are as follows: Class 1, normal stable trajectory; Class 2, High BMI resolving; Class 3, progressively overweight stabilising; Class 4, progressively obese increasing; Class 5, rapidly overweight stabilising obese; and Class 6, overweight/obese persisting.

Appendix 6.7. Risk ratios (RR), 95% confidence intervals (CI) and Wald z-statistic p-values of the associations of BMI trajectory groups with high-risk cardiometabolic outcomes in adulthood after further adjustment for family history of each outcome and for socio-economic status.

Outcome and Latent BMI Trajectory group	RR[c]	95% CI[c]	P-value
Class 1[a]	1[b]	-	-
Class 2	1.93	0.11 – 9.73	0.40
Class 3	2.09	1.09 – 5.11	**0.02**
Class 4	10.1	3.13 – 19.72	**0.03**
Class 5	9.33	4.12 – 16.15	**0.002**
Class 6	16.5	6.30 – 22.61	**0.01**
Hypertension			
Class 1[a]	1	-	
Class 2	0.52	0.13 – 1.32	0.15
Class 3	1.24	1.11 –1.99	**0.04**
Class 4	2.12	1.15–2.89	**0.02**
Class 5	2.28	1.32–3.02	**<0.01**
Class 6	2.98	1.51–5.02	**0.03**
High-risk cIMT			
Class 1[a]	1[b]	-	
Class 2	3.12	1.51 –6.03	**0.04**
Class 3	1.31	1.01–2.14	**<0.01**
Class 4	2.19	1.31 –3.90	**<0.01**
Class 5	3.10	1.92 –3.45	**0.02**
Class 6	3.14	2.21 –4.12	**<0.01**
High-risk LDL-C			
Class 1[a]	1[b]	-	-
Class 2	1.01	0.1 – 1.08	0.45
Class 3	1.12	1.06 – 1.49	**0.02**
Class 4	1.30	1.17 – 2.57	**0.05**
Class 5	1.20	1.11 – 2.30	**0.03**
Class 6	1.51	1.05 – 2.94	**0.05**
High-risk HDL-C			
Class 1[a]	1	-	
Class 2	1.03	0.22 – 1.11	0.36
Class 3	1.24	1.12 –1.82	**0.03**
Class 4	1.35	1.01–12.1	**<0.01**
Class 5	1.41	1.02 –2.22	**<0.01**
Class 6	1.37	1.26–2.60	**0.04**
High-risk triglycerides			
Class 1[a]	1[b]	-	
Class 2	0.31	0.06 –2.12	0.42
Class 3	2.89	2.02–4.08	**<0.01**
Class 4	5.11	3.11 –9.58	**0.03**
Class 5	4.24	3.02 –7.34	**0.02**
Class 6	3.21	1.22 –9.60	**0.04**

[a]Latent BMI trajectory classes are as follows Class 1, Normal stable trajectory (N=1359); Class 2, high BMI resolving (N=43); Class 3, progressively overweight stabilising (N= 879); Class 4, progressively obese increasing (N= 110); Class 5, rapidly overweight stabilising obese (N= 113); and Class 6, overweight/obese persisting (N=33).

[b]: The normative BMI trajectory class (class 1) is the reference group. Unadjusted models with only the trajectory groups were also fit but estimated dd Ratios (RRs) were not significantly different and the AIC suggested that the sex-and year of birth (YOB) adjusted models fit the data better (data not shown).

[c]The regression coefficients for each latent class, can be interpreted as the changes in relative ratios for belonging to a given class, vs. the reference latent class (class 1). For year of birth (YOB), the coefficients give the change in relative ratios of the outcome for a one unit increase in the predictor variable.

[d]The 95% CI for the relative risks was obtained by log-likelihood profiling of the robust standard errors.

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